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THE ANATOMICAL RECORD

VOLUME 15

AUGUST, 1918–FEBRUARY, 1919

PHILADELPHIA
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JUNE 24

A CONTRIBUTION TO THE STUDY OF THE PATHWAYS OF THE CEREBROSPINAL FLUID AND THE CHOROID PLEXUS

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NINE FIGURES

In experimental work for demonstrating the presence of lymph-vessels in the dental pulp,[1] use was made of vital staining as a supplementary attempt to obtain some histological information regarding endothelial lined perivascular or other lymph-channels. There are observations recorded in the literature which are vaguely suggestive of such a possibility. Of particular interest from this standpoint are statements made by Brass[2] and by Evans,[3] that the endothelial cells lining the sinuses and lymph-channels of lymph-glands are always intensely stained, and by Kiyono[4] that vitaliy stained endothelial cells are found "in the lymph spaces of the interstitial tissue of the lung."

A large number of rabbits and a few dogs and cats were injected intraperitoneally and chiefly intravenously with trypan blue and lithium carmine, and microscopic preparations made from practically all organs. Chief attention was paid to those vitally stained cells, which occur in the connective tissue of the various organs and which have been classified as endothelial cells, reticulum cells, resting wandering cells, clasmatocytes, rhagiocrines, histiocytes, pyrrol cells. The impression with me from

[1] Dewey, Kaethe, and F. B. Noyes, "A Study of the lymphatic vessels of the dental pulp." Dental Cosmos, 1917, 58, 436.

[2] Brass, "Über physiologische Pigmentablagerung in den Kapillarendothelien des Knochenmarks." Arch. f. mikrosk. Anat.,[7]1913, 81, 61.

[3] Evans, "The macrophages of mammals," Amer. Journ. of Phys., 1915, 37, 242.

[4] Kiyono, "Die vitale Karminspeicherung. Jena, 1914.

THE ANATOMICAL RECORD, VOL. 15, NO. 1.
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these studies was that these cells are intimately related to the lymphatic system. The regularity with which they occur in the same regions within the organs, the apparently systematic arrangement in which they present themselves, the definite course which so often they seem to pursue, are striking features and tend to dispel the impression that they are cells indiscriminately dispersed within the connective tissue, resting wandering cells waiting for a chance stimulus to arouse them to functional activity and locomotion. When the injections have been continued for some time the cells are large and crowded with stained granules in their cytoplasm and their long processes; the latter not infrequently almost touch one another so that nearly continuous cell tracts are formed, running along the blood-vessels and independently. From these observations, in conjunction with studies of the literature on the lymphatic distribution throughout the body, I have been led to suggest the possibility that these vitally stained cells are for the most part endothelial cells lining the lymph-channels within organs and that vital staining may furnish us the means of demonstrating lymph-channels in organs and regions where they are not yet definitely known or are difficult to demonstrate by the usual methods of injections.

As to the central nervous system it has been and still is a much debated question whether the perivascular spaces should be regarded as lymph-vessels, the decision hinging on this point whether they have an endothelial lining. It is a general observation that the favorite location of vitally stained cells within the connective tissue in all organs is the immediate neighborhood of the blood-vessels. It was therefore to be ascertained whether vitally stained cells are also found about the cerebral vessels. With this point in view, the brain with its membranes and the choroid plexus were studied. The dura and pia, and the choroid plexus of rabbits were carefully removed and spread on glass slides; the choroid plexus of dogs and cats was reduced in thickness by the freezing microtome. Of the membranes the dura always presents the greatest abundance of vitally stained cells; it is also one of the tissues in which a definiteness in the arrangement of these cells is pronounced. They form long continuous

tracts, running along the blood-vessels, but more often apparently in entirely independent directions. Two-, three-, and four-cell tracts may run parallel to one vessel, from which they may be entirely separated. Their independent course is also very conspicuous in regions where the blood-vessels form loops; here the blue- or red-cell tracts sweep across them in continuous straight lines. Compared with the enormous supply of these cells in the dura, those of the pia arachnoid are scanty and more slender. There are always areas where there are no cells or only a few here and there, and these are slender and barely visible with the low-power lens. But there are always patches where they are in rather dense collections, are larger and more deeply stained, and from these cell groups lines of cells run in all directions. After lithium carmine injections the number of vitally stained cells always seem increased and the individual cells are larger. Frequently they have very long processes which almost touch one another, and thus very definite tracts are formed which arbitrarily, it seems, follow some of the blood-vessels, while others and especially all the larger vessels are unaccompanied by such cells. The irregular distribution and the presence of cell patches is a prominent feature in all preparations of pia. In the choroid plexus the ependymal cells covering the vascular convolutions are all densely filled with a finely granular stain and form a continuous blue or red layer around the tufts. In the pial connective tissue of these and the velum interpositum there are always numerous vividly stained cells like those in the dura and pia. They do not form absolutely definite cell tracts, nor do they follow the blood-vessels closely; in fact, they are generally at some distance from them. They are quite evenly distributed throughout and anything resembling the cellular patches of the pia does not occur here. In the intensity of the staining, the large number and the distribution of these cells, the velum interpositum and the pial tissue about the tufts resemble more the dura than the pia.

Being inclined to consider the vitally stained cells as endothelial cells lining the lymph-channels and lymph-spaces I suggest that the cell complexes in the pia arachnoid might be looked

upon as the structures described by Weed[5] as arachnoid villi, structures normally present in the arachnoid of animals and man, and through which, according to him, the cerebrospinal fluid is filtered from the arachnoid spaces into the venous sinuses.

In view of the presence of such vitally stained cells in the membranes of the brain and the choroid plexus, clearly indicating the passage through and into them of fluid containing the staining solution, it is the more striking that the brain tissue fails to show any vitally stained cells whatsoever. The only other exception from the general observation is the fetus which also remains unstained. Goldmann[6] explains this fact by the statement that the dye derived from the plasma is filtered off by the cubical epithelial cells of the plexus and the syncytial cells of the placenta and thus kept off from the cerebrospinal and the amniotic fluid. Those two tissues form a protecting limiting membrane to the brain and fetus. Trypan blue, according to him, is a substance extremely toxic to the nervous tissue. He claims that while 50 cc. of a 1 per cent solution may be injected in a single dose into a rabbit intravenously without any untoward effect, ½ cc. of a ½ per cent solution of the stain injected into the spinal or cerebral subarachnoid space produces tonic and clonic convulsions within ten to twenty minutes and death in coma within one or two hours. In these cases he found that the ganglion cells had a diffusely stained cytoplasm and a stained nucleus, indicating that death of the cells had taken place. But he also described genuine vital staining of cells within the connective tissue of the pia and along the vessels of the pial septa. (These cells are called in all his publications 'pyrrol cells,' because he first observed typical granular vital staining of these cells after injections of pyrrol blue.) "The supposition," he writes, "that

[5] Cushing, Weed and Wegefarth, "Studies on the cerebrospinal fluid." Journ. of Med. Research, 1914, 26, 51.

[6] Goldmann, 1 Die aussere und innere Sekretion des gesunden und kranken Organismus in Lichte vitaler Farbung. Beitr. z. klin. Chir., 1909, 54, 192, and 1912, 78, 1.

2 Experimentelle Untersuchungen uber die Funktion der Plexus choroidea und der Hirnhaut. Arch. f. klin. Chir., 1913, 101, 735.

3 Die Vitalfarbung des Centralnervensystems. Berlin, 1913.

the perivascular prolongations of the pial funnels functionate as 'lymphspaces' finds a further support in the fact that the cells which line these spaces, as well as the fine connective-tissue trabecula traversing these spaces, store up our vital stains in fine granules in exactly the same way as the reticulum cells in the lymph glands." These statements by Goldmann induced me to also use subarachnoid injections of trypan blue solutions not only for the demonstration of endothelial lined perivascular lymph-channels in the brain, but as well for some further information about the choroid plexus. However plausible his explanation of its function as a protecting membrane, some questions evolving from such a conception remain still obscure; for example, how is it possible that the filtering process by the cuboidal epithelial cells of the choroid plexus can protect the entire brain against injurious substances from the blood plasma when other vessels beside the choroidal arteries, in fact, the larger number of the cerebral vessels, pass directly into the brain substance? In what manner is the spinal cord protected which has no choroid plexuses?

The clinical results in twenty-four rabbits which I injected intraspinally and intracranially with trypan blue, did not present the grave symptomatic picture which Goldmann describes as characteristic for the rabbit. Symptoms occurred in a number of these animals; but there were no fatal cases, and in several rabbits even repeated injections failed to elicit any symptoms. In the absence of all grave conditions of convulsions and of coma, the results resembled much more those observed in dogs. Concerning the striking dissimilarity of results observed by Goldmann in these two animals, this author remarks himself: "It seems to me very remarkable that in the dog the symptoms of motor irritation elicited by the dye cannot be compared in any way with those in the rabbit . . . above all, those grave conditions of convulsions may be lacking in the dog which are so exceedingly characteristic of the toxic effects of the dye in the rabbit." I am inclined to believe that the symptoms do not arise so much from any toxicity of the staining substance, as that they are due to mechanical and physical factors and direct injuries of the

nervous tissue in the process of the injections, which are not so easily controlled in the rabbit. The most severe paralysis occurred in a control rabbit after the first injection of distilled water; the paralysis persisted, but the animal recovered otherwise and remained well in spite of a second injection of distilled water and two injections of trypan blue.

The injections were made, as a rule, in the lumbar and lower thoracic region; eight rabbits were injected into the cerebral subarachnoid space. After having anaesthetized the animal with ether, the needle was passed through the skin and muscles into the spinal cavity by way of the interspinous foramina, which are rather large in young rabbits. The cranial injections were made through the occipito-atlantoid ligament. I found soon that it was best to use a very fine needle and pass it quickly through the cord in an oblique direction and inject the fluid into the space opposite the point of entrance. The needle's striking the bony wall makes it sure that the fluid is not forced into the nervous tissue, an accident which is not so easy to avoid if the fluid is injected where the needle passes through the interspinous ligament. In injections into the cerebral subarachnoid space some of the fluid was always withdrawn before injecting the staining solution. In larger animals the drops would flow out spontaneously through the medium-sized needle; in very young rabbits a very fine needle was used and here the fluid was aspirated with a medicine dropper. As to the spinal cord, attempts to let some of the fluid escape before injecting the solution were given up as rather difficult. For this reason I cannot claim, in spite of all practical considerations and careful study of the anatomic conditions of the spinal cord in the rabbit, to furnish a positive proof that the staining solution actually passed into the subarachnoid space and not into the epidural space. But the clinical and histological observations in these injections being largely the same as in intracranial injections, the controlling tests of the latter should be sufficient to justify my general statements.

In the following table the distinguishing feature for the results, marked as 'positive' and 'negative,' has been the presence or absence of diffusely stained cells in the membranes or the

nervous tissue. Diffuse staining of the nucleus and protoplasm of the nerve cells indicates a grave impairment of their vitality, and since I claim that this is not the result of any toxic action of trypan blue, but arises from other causes incidental to the injections, the presence of diffusely stained cells is applied to represent 'negative results,' and the presence of vitally stained cells 'positive results.' There is evidence that the cells may recover from the condition in which they are susceptible to diffuse staining. Those rabbits which were killed when serious symptoms arose and persisted for several hours were nearly all 'negative' cases; on the other hand, in instances where the animals presenting grave symptoms were allowed to live and to recover and subsequently were again injected with trypan blue without any untoward effects, the results were generally 'positive.'

TABLE 1

Intraspinous injections

Nervous symptoms of a more or less serious nature with negative results

NUMBER OF ANIMAL	WEIGHT	NUMBER OF INJECTIONS	AMOUNT OF DOSE	CONCENTRATION OF SOLUTION	DURATION OF EXPERIMENT	SYMPTOMS	RESULTS: Positive	RESULTS: Negative
	grams		*cc.*	*per cent*	*days*			
1	700	1	1	⅓		Paralysis of hind legs; spasms of muscles of mastication, excitement		—
2	650	1	1.0	⅓		Severe spinal symptoms; paralysis, motion in a circle from right to left		—
3	1020	4	1.5	½	5	Severe symptoms after the fourth injection due to injury in injecting		—
4	810	3	1.0	1	3	Paralysis of hind legs and slight spinal symptoms		—
5	590	2	0.5	1	2	Transitory slight symptoms		—
6	1130	1	1.0	⅓		Remark: injection was made when the animal was succumbing to ether anaesthesia		—
Total								6

TABLE 2

Intraspinal injections

Grave nervous symptoms with positive results

NUMBER OF ANIMAL	WEIGHT	NUMBER OF INJECTIONS	AMOUNT OF DOSE	CONCENTRATION OF SOLUTION	DURATION OF EXPERIMENT	SYMPTOMS	Positive	Negative
	grams		*cc.*					
7	1487	1	1.5	½	20 hours	Paralysis, excitement, spasms of muscles of mastication	+	
8	1015	4	0.5–1.5	½	5 days	Paralysis and spinal symptoms after the 4th injection	+	
9	1372	4	1.5	½	5 days	Paralysis and spinal symptoms after the 4th injection	+	
Total							3	

TABLE 3

Absence of symptoms with positive results

NUMBER OF ANIMAL	WEIGHT	NUMBER OF INJECTIONS	AMOUNT OF DOSE	CONCENTRATION	DURATION OF EXPERIMENT	SYMPTOMS	RESULTS Positive	RESULTS Negative
	grams		*cc.*	*per cent*	*days*			
10	1380	2	1	½	3	Membranes light	+	
11	1265	5	1	½	9	Membranes very light	+	
12	1070	13	1.0-1.5	¼ to ½	16	Membrances very light. Grave paralysis followed first unsuccessful attempt to inject, without any fluid entering the spinal canal. Complete recovery from this in course of following 16 days of experiment	+	
13	918	4	0.5–1.5	1	4	Membranes light	+	
Total							4	

TABLE 4

Injections into the cisterna magna

NUMBER OF ANIMAL	WEIGHT	NUMBER OF INJECTIONS	AMOUNT OF DOSE	CONCENTRATION OF SOLUTION	DURATION OF EXPERIMENT	SYMPTOMS	RESULTS Positive	RESULTS Negative
	grams		*cc.*	*per cent*	*days*			
14	640	1	0.5	1		Grave symptoms due to impeded outflow from cisterna magna		–
15	550	2	0.5	1	3	Grave symptoms after the second injection	+	
16	620	2	0.5	1	4	Disturbance of equilibrium, transitory	+	
17	730	1	0.5	$\frac{1}{4}$	3	Slight transitory symptoms	+	
18	950	1	0.75	$\frac{1}{4}$	3	Nystagmus; motions of the head	+	
19	1060	3	0.5	$\frac{1}{4}$	5	No symptoms	+	
20	1530	5	1.0	$\frac{1}{4}$	8	No symptoms.	+	
21	1500	1	0.5	$\frac{1}{4}$	4	Direct injury of medulla oblongata; lasting disturbance of equilibrium	+	
Total							8	

TABLE 5

Tests for the comparative effect of distilled water and trypan blue solutions in the same animal

NUMBER OF ANIMAL	WEIGHT	NUMBER OF INJECTIONS	AMOUNT OF DOSE	CONCENTRATION	DURATION OF EXPERIMENT	SYMPTOMS	RESULTS Positive	RESULTS Negative
	grams		*cc.*	*per cent*	*days*			
22	1280	2 aq. dest. 3 trypan	1.0 1.0	$\frac{1}{2}$	9	Serious symptoms and complete and persisting paralysis of hind legs after first injection of distilled water. Otherwise gradual recovery	+	
23	1250	2 aq. dest. 1 trypan	1.0 1.0	$\frac{1}{2}$	7	Paralysis of hind legs after the 3d injection. No other symptoms		–
24	1460	2 aq. dest. 2 trypan	1.0 0.5	$\frac{1}{2}$	7	No symptoms except dulness	+	
Total							3	

TABLE 6

Experiments to ascertain whether 'Körnchenzellen' in lesions such as may occur in intraspinal injections take up trypan blue when this is injected intravenously

NUMBER OF ANIMAL	WEIGHT	NUMBER OF INJECTIONS	AMOUNT OF DOSE	CONCENTRATION	DURATION OF EXPERIMENT	SYMPTOMS	RESULTS
	gms		*cc.*	*per cent*	*days*		
25	1005	6 intrav. 7 intras. aq. dest.	10	1	8 8	No nervous manifestations	No vitally stained cells in nervous tissue at site of injection or otherwise. Blue Körnchenzellen in membranes of brain.
26	980	6 intrav. 12 intrasp. aq. dest.	10 1	1	8 17	No symptoms	No vitally stained cells in nervous tissue except a few along vessels in one restricted region of brain.
27	1240	3 intrav. 1 intrasp.	1 10	1	9	Complete persisting paralysis of hind legs after 1st injection of distilled water apparently due to direct injury	No vitally stained cells or Körnchenzellen in region where needle had been passed, but enormous numbers of such in the membranes of the region.

In tabulating my results I may say: 16 rabbits gave 'positive' results, 8 rabbits 'negative' results. That the greater number of these were obtained during the latter part of the experiment is very probably due to the greater skill in injecting acquired by practice. Three rabbits were killed after one injection because grave symptoms developed; all gave negative results. One rabbit was injected in a dying condition of the effect of ether; the autopsy was made half an hour after death. Microscopically all the cells and fibers of the membranes were diffusely stained. Control rabbits were among the last in the series and had the benefit of the better technique. One of these was paralyzed in both hind legs by the first injection of distilled water. The

paralysis remained, but the animal recovered otherwise and remained well in spite of another injection of distilled water and three injections of trypan blue. It was killed nine days after the first injection. Microscopically the results were positive. Two other control rabbits had each two injections of distilled water and only one injection of trypan blue. There were no unusual symptoms from either of the injections; both gave negative results. In two rabbits the deep cervical glands grossly showed bluish areas and microscopically revealed rather large regions with vitally stained cells. In two other rabbits the kidneys revealed a distinct bluish tint throughout the cortex; microscopically the epithelial cells of the contorted tubules in disseminate areas contained extremely fine bluish granules; only by most careful study with the oil immersion lens could the granular nature of the stained protoplasm be detected. One of these two cases was a rabbit which suffered from an experimentally produced nephritis. Only in two of the negative cases was intense staining of nerve cells of the cord observed comparable to that described by Goldmann. It was due to the direct injection of the staining solution into the nervous tissue. In remoter regions of the cord there was no such intense staining and it was absent in the brain.

The gross appearance of the brain and cord is characteristic and practically the same when the injections have been successful. The membranes on the convex surface of the brain are always light and in the best cases appear almost entirely colorless. The coloration begins at the olfactory prolongation. On the base there is always more or less intense staining, most markedly over the olfactory lobe, the optic and acoustic nerve, the pons and the medulla oblongata. The membranes of the cord are uniformly tinged. After intracranial injections the coloring gradually decreases downwards.

The histological findings reveal peculiarities. If we take those in intracranial injections as a standard, we find, on the average, that the dura is less supplied with vitally stained cells than the pia. Towards the olfactory lobe the number increases enormously; at the same time the cells are larger. In the pia there

may be an overwhelming amount of cells in this region. Generally there are also throughout more cells in this membrane than in the dura. In case of marked hyperemia or pial hemorrhage there is sometimes an amazing number of granular cells and 'Kornchenzellen' in the convex portion of the pia. The thin membrane over the cisterna magna is well supplied with vitally stained cells. The more surprising is the observation that the tissue about the tufts of the chorioid plexus is totally devoid of vitally stained cells. There is also no trypan blue in the epithelial cells themselves. As to the cord, diffusely stained cells are absent, unless the staining solution has been accidentally injected directly into the nervous tissue. There are no vitally stained cells along the intracerebral or intraspinal vessels in nebualt,ger they may occur in regions here and there. Such foci are, as a rule, small in number; sometimes careful search for them will fail to reveal any.

In a few rabbits lesions had been produced in the spinal cord which were followed by destruction of the nervous tissue and immigration of 'Körnchenzellen' in enormous numbers; these cells are all vitally stained. Those which occupy the region of necrosis and necrobiosis lie close together, are large, more or less rounded and besides blue granules contain diffusely stained cell inclusions. The chemotactic influences emanating from this focus have been made visible, so to speak. Throughout the sections cells marked by blue granules swarm from all directions towards this center; they are slender, increasing in size as they approach the field of phagocytic action; they are all in the immediate neighborhood of the vessels and follow the course of their divisions so that through them the distribution of the vessels is well illustrated. There cannot be any doubt in this case but that these 'Körnchenzellen' are derived from endothelial cells lining the perivascular spaces. A singular observation, however, is that as we get away from this region of necrosis, blue stained cells along the vessels are no longer seen. Goldmann also found such phagocytic vitally stained cells in inflammations of the meninges at the place of the injury. They were not only in the subarachnoid meshwork, but could be followed from the pial

funnels far into the cortex. He considers them as normal constituents of the meninges and their intracerebral, intraspinal, and intraneural prolongations.

Some important observations from intraspinal injections of trypan blue are then briefly these: Vitally stained perivascular cells, endowed with the phagocytic properties of 'Körnchenzellen,' are abundant in a wide range about encephalitic foci. Vitally stained endothelial cells about vessels in the cord and brain are not generally in evidence; they may be present about a scanty number of vessels in the cord and are scarcely, if at all, seen in the brain; apparently there is less tendency in such cells to take the stain as we get away from the region of the injection. The staining solution, however, is early carried with the cerebrosinal fluid to the optic, olfactory, and all peripheral nerves demonstrated by the early staining of their dural sheaths; it is conveyed to the deep cervical glands. May we not conclude from these observations that the endothelial cells of the perivascular spaces in the brain and spinal cord have less affinity for the stain than similar cells in the meninges and in other tissues of the body? that some stimulus is needed to arouse them, so to speak, to reaction to the stain? and that the stimulus in these cases come from the local effects of the injections, not from the stain itself as a 'nerve toxin,' but from direct and indirect mechanical injuries to the nervous tissue, from pressure, from disturbances in the circulation? Such an hypothesis is not without a parallel. Taking it for granted that these particular cells are endothelial in nature or origin as admitted by various investigators, it is a well-known fact that this property of vital staining is peculiar only to a certain proportion of this class of cells. The endothelial cells lining the blood-vessels as a whole do not take the stain. An exception to this rule are those of the capillaries and venules of the spleen, of the blood-vessels of the bone-marrow and the hemal glands, and the Kupffer cells, supposed to belong to the capillaries of the liver. As to the lymphatic system vital staining has been observed in the endothelial cells lining the lymphatic sinuses of the lymph glands. An interesting observation in vital staining concerning these organs has been emphasized by

Evans,[7] who writes: "This participation on the part of the endothelium, however, is sharply limited to those definite tracts of it well within the hemal and lymphatic glands. The entering or draining trunks, in the case of the lymph gland do not show any peculiarity on the part of their lining cells, whereas, in the case of the hemolymph glands, nothing is more striking than the abrupt assumption of brilliant dye granules by the endothelium of a venule just as it enters and resolves itself within the gland." I have ventured to suggest[8] that this difference in the behavior of the endothelial cells towards the stain may correspond to a difference of function on the part of the lymph-channels. Those within the organs are collecting vessels, those outside conducting vessels. As to the Kupffer cells, it cannot be said that a decision as to whether they belong to the blood capillaries or to perivascular lymph-spaces has been absolutely agreed upon. There are a number of writers who assign them to the latter. The other organs in which vitally stained endothelial cells of the blood-vessels occur, namely, the spleen and bone-marrow, are curiously enough those in which blood and lymph are in most intimate relationship to each other; the spleen and the bone-marrow are the birthplace of both elements. The hemal glands may be of a similar nature; they are essentially lymphatic structures.

From the differences in the staining phenomena observed in the central nervous system, we may perhaps draw some conclusions regarding the flow of the cerebrospinal fluid and the relationship of the general lymphatic circulation to the central nervous system. It is certainly very singular that the specific connective-tissue cells about the epithelial tufts of the choroid plexus, which invariably are intensely stained after intravenous injections, are absolutely free from any stain after subarachnoid injections. Since this tissue is a continuation of the pia, we should expect to find vitally stained cells in this location, when they are present in abundance within the pia. It would seem from this that the cerebrospinal fluid containing the staining solution does not

[7] l.c. "The macrophages of mammals." Am. Journ. of Phys., 1915, 37, 242.
[8] l.c.

enter channels within the connective tissue of the plexus, but that such channels receive the dye with a fluid from another source, i.e., the plasma or lymph.

It is stated that differences exist in the character of the ventricular and the subarachnoid fluid. Investigations by several authorities also seem to show that there are lymph systems within the cerebral and spinal lymph system as a whole, independent of one another. D'Abundo,[9] Guillain,[10] Marie and Guillain,[11] Orr,[12] and Homen,[13] from the results of injections by various methods, came to practically the same conclusions, namely, that lymph from the general circulation, which passes along the perineural lymph-spaces of the peripheral nerves and enters the nervous system, follows paths within the limits of definite territories. Thus bacteria or their toxins, experimentally or pathologically inoculated into peripheral nerves, are carried along the lymph-stream to the spine and brain in an ascending direction, capable of producing lesions in regions of the central nervous system quite remote from the seat of the original infection. All these writers agree in the statement that the current of lymph ascending the posterior roots and columns is independent from that of the anterolateral columns. Guillain and Marie showed that the lesions in tabes, always limited to the posterior roots and columns, are the result of the direct propagation of the syphilitic virus along the perineural lymph-vessels. They go so far as to call tabes a lymphangitis of the posterior lymphatic system of the spine.

[9] D'Abundo, Sulle vie linfatiche del Sistema Nervosa Centrale. Annali di Nevrologia, 1896, 14, 229.

[10] Guillain, La circulation de la lymphe dans la moelle épiniere. Revue Neurologique, 1899, No. 23, 796.

[11] Marie et Guillain, Les lésions du système lymphatique postérieur de la moelle sont l'origine du processus anatomo-pathologique du tabes. Revue Neurologique, 1903, 11, 49, 103 and 106.

[12] Orr, A contribution to our knowledge of the course of the lymph-stream in the spinal roots and cord. Rev. Neurol. and Psychiatr., Edinburgh, 1903, 1, 639.

[13] Homen, Die Wirkung einiger anaeroben Bakterien namentlich bei Symbiose und aeroben Bakterien, sowie ihrer Toxine auf periphere Nerven, Spinalganglien und das Rückenmark. Arbeiten aus dem pathologischen Institute der Universitat zu Helsingfors, 1905, 1.1.

Accepting such division of lymph systems, we do not necessarily deduce the existence in the brain and spinal cord of conditions entirely exceptional from those in the rest of the body. Apparent deviations from the general laws of anatomy and physiology are not fundamental differences, but variations conditioned by the structural and functional nature of the organs involved. The meningeal spaces are equivalents of lymph-vessels adopting the form most suitably adjusted to the particular requirements of such an organ with such surroundings. Perivascular lymph-sheaths do not only exist in the brain tissue, which is exceedingly sensitive to variations in the blood-pressure, but are also found in such an unyielding tissue as bone. The cerebrospinal fluid differs from ordinary lymph to the same extent that lymph in other parts of the body is modified by the nature of the tissues which it drains. As to the independence of restricted lymph systems within the central nervous system, parallels can be found in other anatomic units. Sicard, in discussing this question, refers to the independence of the subserous lymphatic network of the stomach from such a subserous network of the duodenum.

Following these lines, we may very well assume that after intravenous and intraperitoneal injections the dye enters the cerebral and spinal membranes and the choroidal connective tissue along real lymph-channels, and the endothelial cells lining these channels take up the vital stain as the fluid containing it reaches them. After intracranial and intraspinal injections, the staining solution follows the paths of the ventricular and subarachnoid fluid, and in leaving the cranial and spinal cavities is not only taken up by the venous drainage, but is conveyed along true lymph-channels.

The pronounced affinity for vital stains exhibited by the specific connective-tissue cells of the membranes over the olfactory tract and at the exits of the cranial and spinal nerves probably coincides with a difference in the nature and function of these cells. At the olfactory lobe, where this change is especially striking, we have a transition from the subarachnoid spaces to the lymph-channels of the nasal apparatus. Similar transitions

we may assume to exist at the seat of the arachnoid villi, where the pia-arachnoid channels more or less directly communicate with the lymph-channels of the dura.

The choroid plexus is, according to Goldmann, a protective limiting membrane filtering off from the blood-plasma a substance which acts as a violent toxin to the nervous tissue. He also suggests that there may be protective substances inherent in the granules of the ganglion cells in the dog, which he observed to be sometimes vitally stained, while in the rabbit he only found diffuse staining of the protoplasm and the nucleus of such cells. Providing the necessity of protection to the nucleus of these nerve-cells, we should reasonably assume that the ganglion cells throughout would possess and manifest such protective powers, which, however, was not observed. It would be equally reasonable to suspect that this is a property common to all cells staining vitally, for it is a characteristic of the phenomenon of vital staining that only the granules take up the dye, while the nucleus remains unstained. On the other hand, we know that diffuse staining of the nucleus and protoplasm, the indubitable sign of injury or death of the cell, takes place not only in the cells of the central nervous system, but in those of all organs of the body. It would rather seem that the ability to take up the stain is dependent on definite chemical or physical properties of the constituents of the granules in certain types of cells. These cells have well-defined characteristics in common, which they do not share with other cells provided with granules which are refractive to vital stains. Investigations of the morphological and histological occurrence of lipoids tend to show that the cells capable of storing cholesterol are the same which react to vital staining, and in either case the ability is confined to their protoplasmic granules. In my own experiments with cholesterolized rabbits,[14] I have observed that cholesterol is stored not only in the more or less coarse granules of interstitial cells classified as macrophages, histiocytes, endothelial cells, etc., but also in the exceedingly fine granules of certain epithelial cells, notably those of the kidney.

[14] Dewey, Experimental hypercholesterolemia. Arch. Int. Med., 1916, 17, 757.

In the choroid plexus the lipoid substances are perhaps best demonstrated by the polarizing microscope. The vessels appear as if invested in an outer silver lining; the doubly refractive substances occur in exceedingly fine divisions; they are distributed all through the tissue.

The presence of granules within the protoplasm of certain types of cells has been associated by some writers with secretory functions. It has also been claimed that these granules are surrounded by a lipoid membrane. In the choroid plexus the extracellular droplets of secretion, which are plainly visible in fresh or formalin-fixed specimens, are manifestly enveloped in such a membrane. Nothing is more striking than Sudan III stained preparations of choroid from cholesterolized rabbits. The tissue is riddled with orange-red spheres and circles. Also in rabbits not injected with cholesterol, these yellow and orange colored droplets may be very conspicuous Evidently the lipoid of this membrane is present in a form other than that within the cells; it does not appear as doubly refractive or anisotropic substances under the micropolariscope.

In a recent publication, Sundwall[15] describes granular interstitial cells in the choroid plexus of the ox, which because of their morphological and staining characteristics he places in the category of mast cells and which he believes may be concerned in some type of secretion. But for his statements that he did not observe such cells in the choroid plexus of other animals--rabbits, guinea pig, dog, human I should be inclined to consider them as identical with the vitally staining granular cells which I found, without exception, in the choroid plexus of the rabbit after intravenous and intraperitoneal injections of trypan blue. I also observed them in the few dogs which I examined. Sundwall mentions variations in the number of these cells with different animals. I have occasionally noticed slight differences, the lessened number coinciding with the diminished intensity of staining, especially in the study of the dental pulp; it was generally found in aged animals, but the observations are not num-

[15] Sundwall, The choroid plexus with special reference to interstitial granular cells. Anat. Rec., 1917, 12, 221.

erous enough to warrant the statement that age is responsible for the decrease in the number of the cells.

An assumed function of whatever nature, inherent in the specifically staining granular cells of the connective tissue within the various organs of the body, gives a peculiar interest to this particular tissue; far from being merely a supporting stroma, this tissue would have all the significance of a vital organ. Such a view is expressed by Renaut[16] in the statement that the connective tissue is "like the largest of the glands with an internal secretion which exists in the body of the vertebras, because it keeps the elements ready for immediate action or susceptible to such action at any time, wherever blood-vessels course through the connective tissue." I am inclined to consider these cells as endothelial cells, being in the most intimate relationship to the lymphatic apparatus and playing a more important rôle in this system than that of merely lining the lymph-channels.

SUMMARY

Concerning the results of these experiments the following observations are to be particularly emphasized:

An apparently exceptional behavior towards vital stains is exhibited by the endothelial cells lining the perivascular spaces, or, if the existence of such cells be denied, by specific cells of the perivascular connective tissue within the brain and spinal cord.

Unlike such cells in the perivascular connective tissue in other organs and tissues, they do not habitually take up the vital stain, but do so only under the influence of stimuli from pathological conditions. This lack of affinity for vital stains may be due to a difference in specific functions inherent in these cells. Differences in the behavior of endothelial cells towards the stain are observed with constancy as follows:

1. With reference to blood-vessels, affinity for the vital stain is absent, in general, in the endothelial cells of the inner lining

[16] Renaut, "Les cellules connectives rhagiocrines." Arch. d'anat. microscop., 1907, 9, 495.

of arteries, veins, and capillaries; present *a*) in the capillaries and venules of the spleen, the capillaries of the bone-marrow and the blood sinuses of the hemal glands, i.e., in tissues the source of blood and lymph elements. *b*) in the Kupffer cells of the liver, i.e., in cells of which it is not yet absolutely certain whether they belong to the capillary wall proper or to the perivascular lymph-spaces.

2. With reference to lymph-channels, affinity for the vital stain is absent in general in the endothelial cells lining the inner wall of lymph-vessels outside the organs; present in lymph-channels within the organs except the brain and spinal cord.

3. With reference to the central nervous system, affinity for the vital stain is absent in general, in the perivascular spaces within the brain and spinal cord; present in these conditionally and in focalized distribution in the presence of pathological stimuli, in general, within the membranes along channels conveying lymph or cerebrospinal fluid.

EXPLANATION OF FIGURES

Fig. 1 Dura. (Intravenous injections of trypan blue; blood-vessels filled with carmine gelatin.) Tracts of vitally stained cells running along the blood-vessels and also in independent directions. Magnification 100 diam.

Fig. 2 Pia-arachnoid. (Intravenous injections of lithium carmine; blood-vessels filled with Berlin blue gelatin.) Two complexes of vitally stained cells; more or less distinct lines of granular cells radiating from these centers. Magnification 100 diam.

Fig. 3 Choroid plexus. (Intravenous injections of trypan blue; blood-vessels filled with carmine gelatin.) The numerous vitally stained interstitial cells are quite evenly distributed; there is no distinct perivascular arrangement Magnification 100 diam.

Fig. 4 Dura. (Injections of trypan blue into the cisterna magna Region of transition from the brain to the olfactory tract. Tracts of vitally stained granular cells closely following the blood-vessels. There was a striking lack of vitally stained cells in the dura over the hemispheres. Magnification 100 diam.

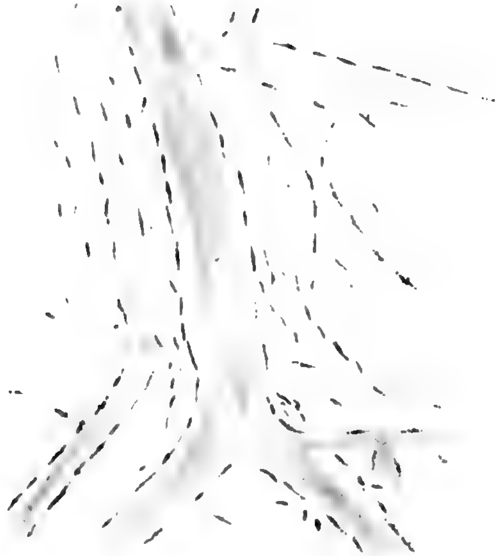
Fig. 5 Intracerebral blood-vessel. (Injection of trypan blue into the cisterna magna.) Perivascular vitally stained granular cells. Magnification 100 diam.

Fig. 6 Spinal cord. (Intraspinal injection of trypan blue.) Enormous accumulation of 'Körnchehzellen' in a region near the central canal Tracts of slender granular cells converging towards the focus; they follow the course of the blood-vessels and increase in size as they approach this center. Photomicrograph. Magnification 135 diam.

Fig. 7 Choroid plexus. Stained with Sudan III and hematoxylin. Extracellular droplets of secretion with a clear center and a lipoid membrane. From an apparently normal rabbit. Magnification 100 diam.

Fig. 8 Choroid plexus. From a rabbit injected with cholesterol. Globular and ring-shaped extracellular droplets of secretion; stained with Sudan III; the tissue is very faintly stained with hematoxylin. Photon icrograph. Magnification 275 diam.

Fig. 9 Pia-arachnoid. (Intraspinal injection of tryp in blue.) Diffuse staining of cellular elements and all the fibers of the connective tissue demonstrating the complex structure of this membrane.



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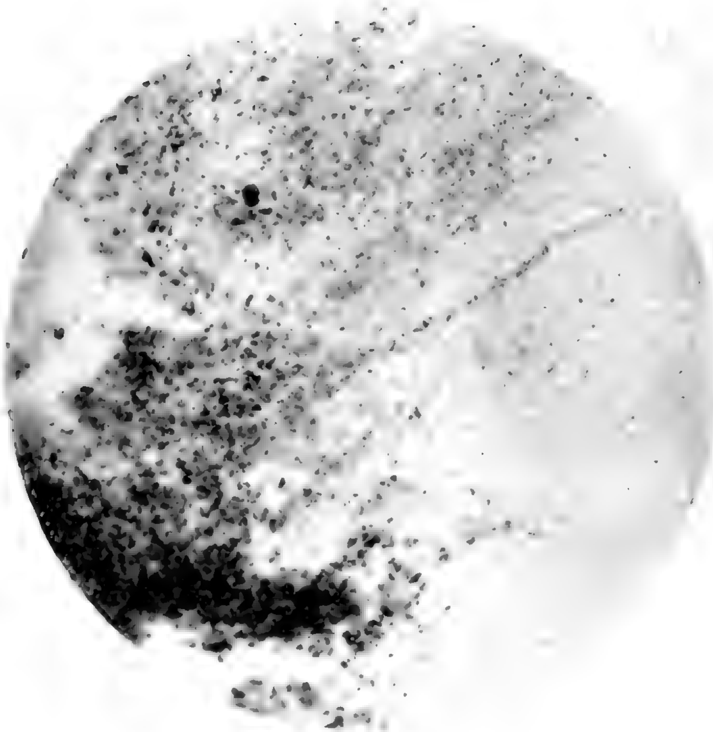

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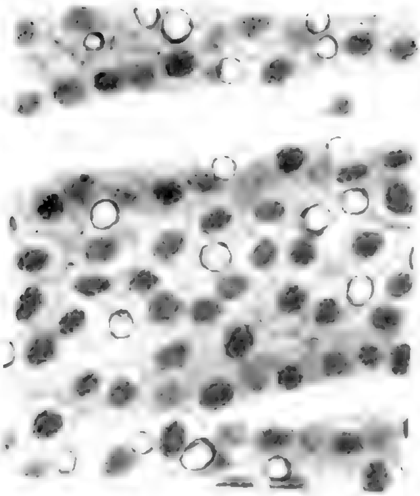

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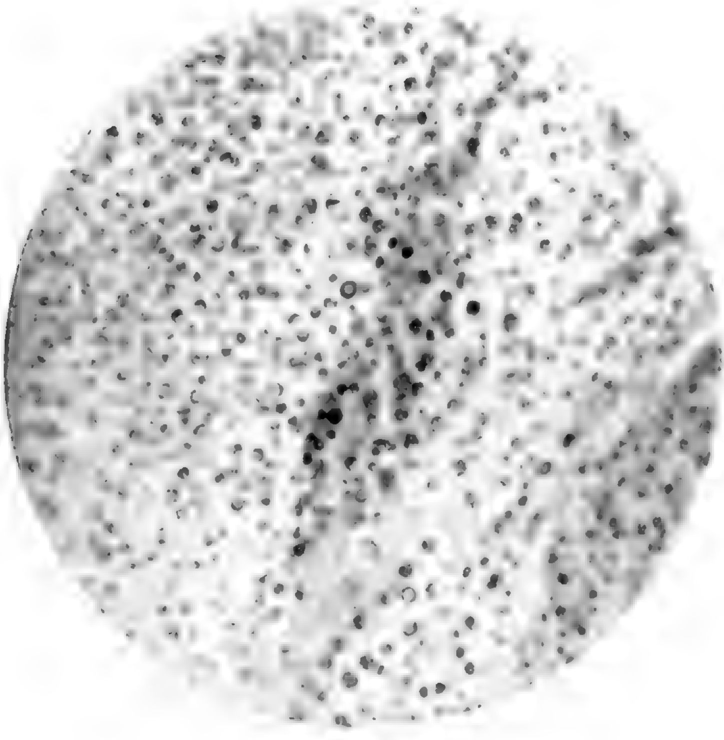

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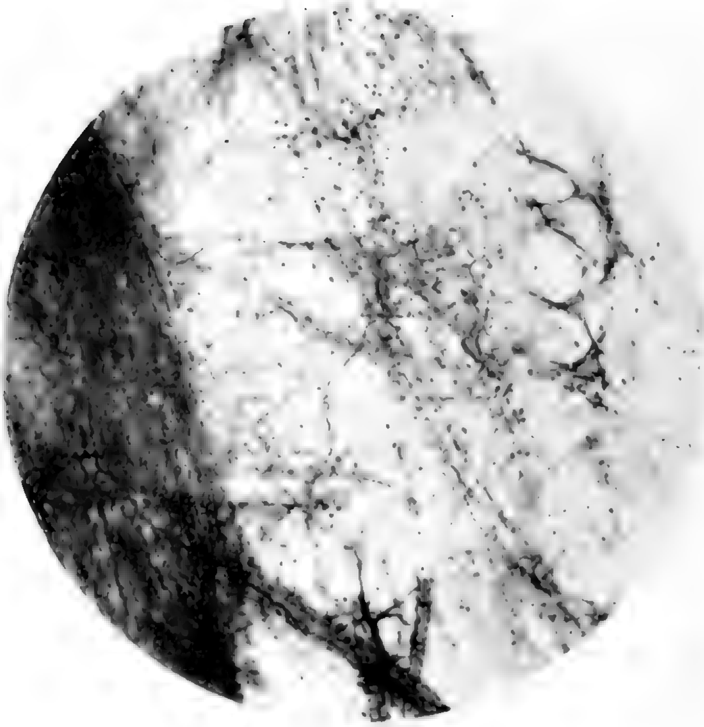

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JULY 19

A DESCRIPTION OF A CASE OF FALSE HERMAPHRODITISM

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ONE FIGURE

My interest in this case was first aroused because the autopsy materials included, besides two abdominal testes, a bilateral pair of symmetrically placed small abdominal bodies suggestive of ovarian remnants. These bodies proved on microscopical examination to be altered lymph-nodes, such as are typical for certain inflammatory conditions. The nodes are characterized by fibrosis, hyalinization, and arteriosclerosis. The case is therefore one of masculine pseudohermaphroditismus, and is consequently of relatively lesser importance. However, since the microscopical data in regard to the testes have a bearing on the alleged causal relationship between the interstitial cells and the development and maintenance of the male secondary sexual characters (Bouin et Ancel[1]), as also on the hypothesis that 'heat' is dependent upon an internal secretion on the part of these cells, a detailed description of these organs seems warranted. In view of the wide generalizations current, as compared with the relatively meager body of definite data, concerning the function of the interstitial cells, it would seem that any additional relevant microscopical observations should be carefully recorded.

The subject was a patient of Dr. C. S. Underhill, of McGaheysville, Va., who first directed my attention to the case. The autopsy was performed on October 31 by the coroner of Rockbridge County, Dr. J. M. Biedler, of Harrisonburg, Va. The

[1] Bouin et Ancel, 1904. Recherches sur la signification physiologique de la glande interstitielle du testicule des mammifères. Jour. de physiol. et de path. gen., T. 6, p. 1012.

internal and external genitalia were preserved in 10 per cent formalin. I am greatly indebted to both of these gentlemen for their interest in this case and for kindly sending me well-fixed tissues and full autopsy notes.

Dr. Underhill's notes include the following pertinent data: The subject was thirty years of age, white, of good physiognomy, with narrow forehead, high cheek-bones, and only a thin growth of hair on top of head. He had supernumerary fingers and toes, the four additional digits being accessory to each of the fifth

Fig. 1 Photograph of masculine false hermaphrodite, with polydactylism and talipes varus. (Kindness of Dr. C. D. Underhill.)

members. Both feet were clubbed (talipes varus). The mammary glands were well developed, the penis was rudimentary, the scrotum was vestigial, and the hands, hips, thighs, and legs were of female conformation. There was much fat everywhere; all organs were covered and infiltrated with fat; the subcutaneous fat was 4 inches thick. Opposite the internal ring on each side was a mass having the 'feel of ovary or testicle.' Two undescended testicles were removed from a sort of canal external to the internal ring, from which the finger could be pushed down into a 'pouch of skin where the scrotum should have been.'

On subsequent inquiry the following additional facts were ascertained: The subject was 'very feeble-minded,' left-handed, unmarried, with no tendency to 'venery.' His parents were 'about third cousins.' His mother had a severe case of heat prostration at the age of 15 to 17; she was in poor health when married at 22. She has been married twice; her one child, a daughter, by her first husband is 'alive and well.' She had three children by her second husband; one died in infancy, and a feeble-minded daughter, sister of the subject, is still living; 'she is able to work about the house.' The parents deny any abnormalities; they do not have supernumerary digits.

The specimen of the external genitalia shows only a short penis, of 2.5 cm. length. The internal, or hidden, portion of the penis has a length of 9.5 cm. Taken as a whole, this organ is therefore about two-thirds average length, and not actually, as apparently on external view, 'rudimentary.' The scrotal region is marked by a small area of pigmented, loose, greatly wrinkled skin.

The bodies originally suspected of being ovaries were encased in an envelope of fat, about 6 mm. thick. The larger measured 3.5 x 2 x 0.5 cm.; the smaller, 2 x 1.25 x 0.5. The general shape was flattened reniform. The central core of the larger body, apparently of connective-tissue structure, was triangular in transverse section, measuring 8 mm. from base to apex and 5 mm. along the base; the latter was indented by a hilus.

The central cores only of both of these bodies were embedded in paraffin, sectioned at 5 microns, and stained with iron-hematoxylin followed by van Gieson's stain. The preservation was superb. The bodies are unmistakably lymph-nodes, in which the reticular tissue has increased enormously. Approximately half the volume of these nodes consists of dense hyalinized reticulum. At first sight this appears homogeneous, but closer inspection reveals a fine fibrillar constitution. The larger denser areas occur in the cortex, apparently at the centers of the original nodules, and along the medullary cords. The reticulum of the denser masses shades off into the loose reticulum of the medullary sinuses. The lymphocytes are well preserved and appear normal.

The testicles have a flattened oval form, roundly triangular in transection. They differ greatly in size: the larger measures 5 x 2.5 x 1 cm.; the smaller, 2 x 1.5 x 1 cm. Microscopical examination shows that they are atrophic, similar in appearance to undescended testes, but with the usual degenerative phenomena greatly accentuated, the degree of abnormality corresponding with the size difference. The sections were stained with the iron-hematoxylin van Gieson's combination. The tunica albuginea is greatly thickened. The testes are characterized in general also by fibrosis and arteriosclerosis.

Seminiferous tubules are still distinguishable in certain regions of the larger testis, but their tunica propria is greatly thickened and converted into a fibrous hyaline wall. Throughout this wall nuclei of variable form, size, and staining capacity are sparsely scattered. The nuclei are relatively small; they have an oval, bilobed or irregularly lobed form, suggestive of amitotic division. The larger oval nuclei are generally vesicular; the smaller and more irregular forms are usually pyknotic. The perinuclear region, corresponding to the cytoplasmic area, appears clear and homogeneous, the tissue as a whole thus resembling somewhat fibrocartilage. The surfaces of the wall have become sinuous or corrugated through contraction. The thickness of the wall varies as the diameter of the cross-sections, the smaller tubules generally having the more robust walls. In certain regions of the larger testis the tubules have collapsed and become solidified into cords of hyaline material.

In the smaller testis practically all of the tubules have become solidified. These denser, tubular, areas are separated by narrow areas of hyalinized fibrous tissue with few cells containing mostly pyknotic irregular nuclei, and an occasional small degenerating interstitial cell. All of the intratubular lining cells have disappeared in the smaller testis.

The patent tubules of the larger testis contain a peripheral layer of irregularly columnar or polyhedral cells, one to three layers thick. It seems most probable that the lining epithelium was originally single-layered and that this became 'stratified' through a dislocation of certain cells following the shrinkage of

the tubules during the process of hyalinization of their walls. Certain of the smaller tubules are filled with cells arranged in the form of a reticulum. The reticulated appearance is due largely to a vacuolization of the cytoplasm. Certain cells contain a single large spherical vacuole, the nucleus having been pushed to one pole, giving these cells the appearance of fat cells.

The nuclei of the less degenerate cells are relatively large, oval, and vesicular, and contain a delicate reticulum; those of the more extensively vacuolated and irregular cells appear collapsed and pyknotic. In the larger tubules an occasional cell may be found of spheroidal shape with large vesicular nucleus suggesting a spermogonium. Besides these very infrequent cells, the lining cells include no elements which resemble typical spermogonia or spermocytes; they are most probably largely remnants of Sertoli cells and possibly in very small part of primordial germ cells. Between the tubules occurs a coarsened fibrous connective tissue with numerous small, irregular, more generally pyknotic, nuclei. Relatively few scattered interstitial cells are discernible.

The chief point of interest in the case centers on these interstitial cells, considered in relation to the mixture of secondary sexual characters and the absence of sexual desire in the subject. The interstitial cells were identified by comparison with those of a normal testis, the spermogenesis of which was previously described.[2] Compared with those of the normal testis, the interstitial cells of this hermaphrodite are very few, much smaller, approximately half the normal size, and the cytoplasmic content of lipoid granules, instead of being scattered, is grouped into one or several large clumps. The cells could be studied best in unstained preparations. Thanks to their brownish-yellow granules they could be easily detected. They are widely scattered; they have a spheroidal or polyhedral shape; certain have a large, oval, vesicular nucleus, but the majority contain only a small, frequently irregular and pyknotic, nucleus.

[2] Jordan, H. E., 1914. The spermatogenesis of the mongoose; and a further comparative study of mammalian spermatogenesis, with special reference to sex chromosomes. Pub. 182, Carnegie Institution of Washington, p. 163.

The microscopical evidence shows, then, that these testes contained originally both the intra- and intertubular cellular elements characteristic of normal fetal male gonads, but that both had suffered a gradual degeneration, probably in part pathological, until only modified intratubular, chiefly sustentacular, cells and degenerate interstitial cells remained throughout the hyalinized stroma. The persistence of even a few recognizable interstitial cells under the conditions which obtained in these testes, even in the more atrophic of the two, indicates a most remarkable vitality and suggests an important rôle on the part of these cells.

The case under consideration appears to be one of arrested male development. All things considered this pseudohermaphrodite is very decidedly more male than female. The female secondary sexual characters are superimposed on a male substructure. And it may be emphasized that the 'female' characters are not infantile; witness the well-developed mammary glands. Nor are they wholly dependent upon the considerable adiposis. For some reason certain female secondary sexual characters have gained formal expression in this essentially male individual. The underlying proximate cause is most probably the atrophy of the testes. The ultimate cause would seem to have been one common to the hermaphroditism and the concomitant physical (supernumerary digits, club-foot) and functional (left-handedness; feeble-mindedness) anomalies. In respect to this congeries of anomalies, presumably dependent upon a common germinal factor or disturbance, the case is practically a duplicate of one previously described,[3] where a very similar pseudohermaphrodite was one of nine children, all of whom showed various anomalies, including polydactylism and gigantism, and adds another to a large and variable group of pseudohermaphroditic malformations coincident with other minor anomalies.

Thus the 'rudimentary' penis, vestigial scrotum, and cryptorchism appear to be different aspects of a general anomalous condition. The case may be conceived to have developed as

[3] Jordan, H. E., 1912. Studies in human heredity. Bull. Philosophical Society, Univ. of Virginia, Scientific Series, vol. 1, no. 12, p. 293.

follows: The atrophy of the testes, and the suppression of the scrotum, supervened upon the inguinal retention of the testes; the development of certain female secondary sexual characters resulted from the atrophy. The effect of the original cryptorchism may have been modified by pathologic factors, for undescended testes commonly have an increased number of interstitial cells coincident with, and reciprocal to, a decrease in the number and activity of the intratubular spermogenic cells. It would be of interest to know in this connection also the condition of the hypophysis, but this organ was not removed at autopsy.

The well-attested lack of sexual desire in connection with the paucity and abnormality of the interstitial cells is perhaps the most important phenomenon pertaining to this case. That the control of the male secondary sexual characters is not dependent upon the interstitial cells of the testis seems proved by this case, where a mixture of secondary sexual characters is maintained in the substantial absence of healthy interstitial cells. Such conclusion must be drawn also from the various castration and spaying experiments where certain secondary sexual characters, generally reciprocal, originate and persist in the absence of interstitial cells. The same conclusion is indicated also by conditions in certain turtles which lack secondary sexual characters and where the male nevertheless possesses abundant interstitial cells during the spring, for a period probably corresponding with the breeding season.

The hypothesis that 'heat' is dependent upon the integrity of the interstitial cells seems to rest upon a firmer basis of observational data. The case described by Whitehead,[4] of a stallion with a third abdominal testis, seems conclusive on this point. The whole mass of data relating to infertile hybrids (e.g., mules) and cryptorchid individuals supports the same conclusion. For here heat remains in the absence of potency, correlated with a numerical increase of interstitial cells and an ab-

[4] Whitehead, R. H., 1908. A peculiar case of cryptorchism, and its bearing upon the problem of the function of the interstitial cells of the testes. Anat. Rec., vol. 2, p. 177.

sence of complete spermogenic activity. The hermaphrodite here described adds similar evidence, though of negative character. Here heat was absent, a condition correlated with few interstitial cells and these of degenerate character.

The sufficiency of this latter hypothesis, as well as the now more generally discarded earlier one of Bouin and Ancel, is controverted by Boring and Pearl[5] on the basis of their results from a microscopic study of the testes of chickens, in individuals of which of over six months they claim interstitial cells are wholly lacking though of 'full sexual normality both in respect of primary and secondary characters' (p. 265), These results, however, are at variance with those obtained by the recent careful work of Reeves,[6] who reports interstitial cells in the testes of chickens at all ages up to eighteen months.

The coincidence of left-handedness in this case with various anomalies (polydactylism, rudimentary and atrophic genitalia) supplies additional interest. In my studies of hereditary left-handedness[7] I have been led to adopt the hypothesis that the condition follows a fetal structural variation in the cerebral blood-supply, of a nature to effect a better nutrition of the right cerebral hemisphere in contrast to the usual condition where the left hemisphere is better supplied. An examination of the fetal vascular system suggests that the arrangement of the common carotids, innominate and subclavian arteries is such as to favor the possibility of a larger blood supply to the left cerebral hemisphere and the right arm, thus presumably determining a bias towards right-handedness. If this morphologic explanation of right-handedness and left-handedness is correct, then it is significant that also left-handedness should occur in this case where various other anomalies are associated with hermaphroditism.

In view of these data the conclusion is suggested that a condition of pseudohermaphroditism is of the same category as the

[5] Boring, Alice M., and Pearl, Raymond, 1917. Sex Studies. IX. Interstitial cells in the reproductive organs of the chicken. Anat. Rec., vol. 13, p. 253.

[6] Reeves, T. P., 1915. On the presence of interstitial cells in the chickens' testes. Anat. Rec., vol. 9, p. 383.

[7] Jordan, H. E., 1914. Hereditary left-handedness, with a note on twinning. Journal of Genetics, vol. 4, p. 68.

common anomalies of other systems. The accompanying functional disturbances in this case, expressed in loss of sex potency and subsidence of 'desire,' resulted from the induced atrophy following the inguinal retention and probably later disease of the testes. Whether subsidence or accentuation of heat follows cryptorchism seems to depend upon whether the resulting testicular atrophy involves also the interstitial glandular cells or only the seminal epithelium.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JULY 19

THE HISTOLOGY OF LYMPH, WITH SPECIAL REFERENCE TO PLATELETS

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The primary object of this investigation is to determine whether blood-platelets occur in lymph. The material employed consists of stained smear preparations of lymph from the thoracic duct of the dog, treated according to Wright's method for blood smears. The desirability for further studies on the structure of lymph arises by reason of the discrepancies in the results reported by recent investigators. Schäfer ('12) makes the unqualified statement that "both lymph and chyle contain thrombocytes" (p. 394). Davis and Carlson ('09) in their study of the cell-content of lymph, make no mention of platelets, permitting the inference that they did not see these elements in lymph. Vinci and Chistoni ('10), on the basis of careful microscopic studies of the lymph, of dog, cat, and rabbit, conclude that lymph lacks platelets (p. 209). Howell ('14) comes to the conclusion that "platelets do not occur in lymph from the thoracic duct of the dog."

The following general facts would lead one to expect to find platelets in lymph: 1. Platelets play an important part in the clotting of blood "by liberating an additional supply of thromboplastic substance and of prothrombin" (Howell, '14). Lymph, in common with blood, contains fibrinogen and thrombin, and under similarly favorable conditions clots promptly. Since thrombus formation in blood is apparently largely dependent upon the presence of platelets, the latter would be expected to occur also in lymph, which has a similar physical and chemical constitution and clots like blood under similar conditions. 2. With the possible exception of platelets, lymph contains the same cellular elements, in vastly different proportions, as blood. If any bar-

rier between the blood-vascular and the lymph-vascular systems can be passed by all the other cellular elements of the blood, even the erythroplastids, it seems reasonable to suppose that platelets also would not be excluded from lymph. 3. The origin of blood-platelets from segmenting pseudopods, and from fragmenting larger areas of cytoplasm, of a certain type of megakaryocyte in the red bone-marrow is well established (Wright, '10; Bunting, '09; Downey, '13; Jordan, '18). Since the platelets pass readily into blood-capillaries of the marrow, no plausible explanation presents itself as to why they are excluded from the lymphatics of the marrow.

The recent work of Howell ('14) is the most thoroughgoing that has yet been devoted to the question of platelets in lymph. It is required, therefore, that this work be first carefully analyzed, with a view to determining whether any possible contingency remains which has not been fully met in this search for the possibly very elusive platelets in lymph. Such a contingency would serve as the only excuse for any addition to an otherwise apparently conclusive research.

Howell's investigation of platelets in lymph is part of a larger research relating to the mechanism of coagulation in blood and lymph. He obtained the lymph of his experiments from dogs anesthetized with morphia and ether, by placing a vaselined cannula into the thoracic duct. The rate of flow was variable, but averaged about 0.5 cc. per minute. The lymph from dogs starved for forty-eight hours clotted in from ten to twenty minutes. The more milky lymph from fed animals clotted in from thirty to sixty minutes in the case of earlier specimens; lymph collected after an hour or two only clotted after from one to three hours. Howell searched for platelets both in the fresh and in oxalated lymph of these experiments. He examined also the corpuscular sediment obtained by centrifugalization of oxalated lymph, but neither in the deposit from a first nor in that of a second centrifugalization could he discover platelets, though the same method applied to blood-plasma revealed them in great abundance. Howell ('14) concludes that "platelets do not constitute a normal element in the lymph."

According to Howell, clotting involves the coöperation of four constituents: 1, fibrinogen, in solution in plasma and lymph; 2, prothrombin, liberated by platelets and by lymphocytes; 3, antithrombin, present in lymph and in blood-plasma in substantially equal amounts, and 4, thromboplastin, liberated by platelets and lymphocytes and tissue cells in general, and operating to neutralize the antithrombin. Pure lymph uncontaminated with tissue juices is said to coagulate more or less imperfectly compared with mammalian blood, but it coagulated promptly and firmly if tissue extract is added to it. Howell explains the inferior clotting of lymph as due to the comparative paucity of thromboplastic material, the result of the absence of the less stable platelets and the consequent restriction of its source to the relatively more stable lymphocytes. This explanation is in accord with the earlier conclusion of Vinci and Chistoni ('10) that coagulation can occur in the complete absence of platelets. These authors concluded, moreover, that "the principal morphological blood element intimately related to the phenomenon of coagulation is the white corpuscle" (p. 212).

In view of the facts that general considerations relating to the comparative microscopic structure of lymph and blood, and to the mechanism of clot formation as formulated by Howell, seemed to favor the probability that lymph contains platelets as expressly claimed by Schäfer ('12), it occurred to me that failure on the part of Howell, and of Vinci and Chistoni, to discover the alleged elements might be due to some unfavorable factor in their technical procedure with reference to platelets. It is well known that the leucocytes in the blood-vessels tend to collect peripherally. Also the extreme adhesiveness of the platelets and leucocytes in general is obvious. It seemed possible that the slow flow of the lymph (0.5 cc. per minute, Howell) might permit the elimination of platelets from specimens collected by cannula from the thoracic duct, by reason of their adhesion to the wall of the duct. A final link in the chain of apparently complete evidence respecting the absence of platelets in lymph as given by Howell, seems to demand an examination of lymph in the form of specially prepared smears made directly from the thoracic duct.

Such hypothetical demand furnished the reason for the present investigation and suggested the special technic employed.

This investigation includes lymph smears from two different dogs prepared at an interval of several weeks. Control specimens of blood were made from these same animals. The technical procedure, for assistance in which I am greatly indebted to Dr. J. A. Waddel, professor of Pharmacology, at the University of Virginia, was as follows: The first dog was quickly killed with ether. The thoracic and abdominal cavities were immediately opened, and a segment of the thoracic duct tied off and excised. The segment was thoroughly rinsed in physiologic salt solution at body temperature, to cleanse it of any possibly adherent blood. A cut was promptly made into this segment of thoracic duct and the lymph content allowed to flow onto slides. The lymph had a clear, watery appearance. Subsequently the segment was cut along the entire length and slides were smeared from the inner surface of the duct. The slides were at once treated according to Wright's staining technic. Control blood smears, stained according to the same method at the same time, showed abundant platelets. But the lymph smears apparently lacked platelets completely.

I was at first surprised to find erythroplastids among the cellular content of the lymph. I suspected that their presence was the result of the death struggle, they having possibly been sucked back from the subclavian vein. However, if their presence was to be accounted for on this basis, typical platelets should occur for the same reason. I find that Howell, and Davis and Carlson, also record the presence of a certain small number of red corpuscles in lymph. In the second experiment almost immediate death was caused by administration of chloroform by mouth, with the idea of obviating any possible reverse flow from the subclavian vein at death. But the preparations from this specimen also showed the presence of a few red corpuscles. Accordingly, we may conclude, I believe, that erythroplastids are a normal constituent of lymph. Nor did any of this second series of slides reveal typical platelets.

The question arises as to how the presence of the erythroplastids in lymph is to be interpreted. Davis and Carlson ('09) only regard the presence of erythroplastids as due to blood admixture (p. 16). In accord with the monophyletic theory of blood-cell origin, such red corpuscles might represent differentiations of certain lymphocytes, serving as hemoblasts, in their slow passage through the lymphatic tree. However, if this conclusion were correct, one should probably expect to find also erythroblast stages; but such could not be detected in my slides. It seems perhaps more reasonable to conclude that the few erythroplastids present in lymph have their origin in the red marrow drained by certain perivascular lymphatic terminals of the system. This conculsion, however, requires that a consistent answer be given to the question as to why platelets, which theoretically could enter the system in the same way, cannot be found in lymph from the thoracic duct. It seems altogether probable that platelets do actually enter the marrow lymphatics in small numbers. The lymph of the thoracic duct contains elements generally liberated by platelets, namely, prothrombin and the thromboplastin (Howell), but in relatively smaller amounts than found in blood-plasma. Platelets are notoriously unstable structures. In their relatively slow passage (compared with the blood circulation) through the lymphatics to the thoracic duct, the relatively small number that may have entered the system may have entirely disintegrated, meanwhile contributing the relatively small amount of prothrombin and thromboplastin characteristic of lymph; to which the lymphocytes under favorable conditions may contribute a certain additional amount, as suggested by Howell. Considering the origin of the bulk of the lymph from the plasma via the tissue spaces, and the continual disintegration of blood-platelets, the presence of these elements (thrombin and thromboplastin) in lymph can be accounted for at least in part on the basis of this relationship between blood plasma and lymph. Similarly with respect to the thromboplastin of the tissue cells.

Starling ('15) inclines to regard blood-platelets as precipitation products in plasma, following contact with foreign bodies or the lowering of its temperature from 37°C. to 18° (p. 837). He

bases his opinion on the observations, following Buckmaster: 1. When a film of blood, held in a platinum loop and kept at body temperature, is examined under the microscope, no platelets can be seen; on cooling, platelets make their appearance. 2. When noncoagulable plasma, e.g., peptone plasma and oxalate plasma, is kept for twenty-four hours at 0 C., after removal of all formed elements by centrifugalization, a precipitate forms 'indistinguishable from blood-platelets.'

With regard to the first observation it may be justly urged that the fact that the platelets are not microscopically visible in the platinum-loop preparations at body temperature is not conclusive proof that such are not actually present. The refractive index of the platelet protoplasm at body temperature may be so close to that of blood-plasma as to render the platelets practically invisible; on cooling, the refractive index of the platelets may change to a point where they become visible against the background of the fluid plasma.

In opposition to the deductions from the second set of observations, stand the results of the present investigation, namely, that in the smear preparations (essentially of lymph which was allowed to cool and thus suffered coagulation and precipitation of certain proteids) certain platelet-like bodies formed which at first appeared indistinguishable from genuine blood-platelets. However, a careful study, as described in detail below, showed that these bodies are quite different structures, essentially compound precipitation products. Moreover, considering the chemical and physical similarity between blood-plasma and lymph, and in large part their common origin, it seems strange that genuine platelets should precipitate only from the former and not from the latter. And in addition, the fact should again be emphasized that the original results of Wright ('10) regarding the megakaryocyte origin of platelets have been confirmed by at least three subsequent independent workers (Bunting, Downey, Jordan) and his conclusions may therefore confidently be regarded as firmly established. Continued skepticism regarding the genesis of platelets as first described by Wright can only rest, it seems to me, on a failure to repeat in detail Wright's technic in the study of red marrow.

In view of Starling's interpretation of platelets as precipitation products, it becomes important to give in detail the steps by which I reached the conclusion, on the basis of my microscopic preparations, that the lymph from the thoracic duct of the dog lacks platelets completely. The slides were made a year ago and were at once carefully studied. Numerous structures were seen which simulated platelets. The possibility seemed to present itself that these bodies were modified or disintegrating platelets. The investigation was temporarily abandoned with the idea that the material at hand did not permit of definite conclusions. I subsequently again studied the slides, and again arrived at a point of uncertainty. A third, more intensive effort, has brought me to certain and definite conclusions: Platelets do not occur in lymph of the thoracic duct; the half-dozen bodies in my preparations regarding which some doubt may remain do not materially alter the precision and generality of this conclusion; various and numerous precipitation products appear which simulate platelets, but which are clearly of different origin and of compound structure. This material accordingly yields additional and significant evidence for the disproof of the conclusion that platelet-like bodies in peptone and oxalate plasmas are genuine platelets.

The stained smear preparations of lymph have a background of a homogeneous or very finely granular vacuolated substratum, ranging in color, depending upon a varying density, from a faint pinkish-blue to a light lilac color. Among the spherical vacuoles may occasionally be seen small spheroidal or oval areas of more condensed substratum. Throughout this substratum are scattered the lymph cells. These are much more abundant in the specimens from the first dog, a difference depending, as Howell has shown, upon nutritive conditions, that is, whether the animal was starved or recently fed. The small lymphocytes predominate, forming at least 90 per cent of the entire cell-content. Large lymphocytes are also relatively abundant; similarly large mononuclear leukocytes with reniform lilac-colored nucleus, and a wider shell of faintly pink cytoplasm. Occasional neutrophils, eosinophils, and erythroplastids also occur, probably of marrow

and tissue-space origin. Occasional endothelial cells, detached from the duct wall in the process of making the smears, also occur. The large mononuclear leukocytes are in the vast majority of cases distorted or fragmented. These cells are evidently much more adhesive and fragile, both in respect to cytoplasm and nucleus, than the small lymphocytes. In the close vicinity of such broken leucocytes clumps of fibrin-fibrils occur, suggesting that these cells are more favorable regions for thrombus formation.

Scattered throughout the substratum are also innumerable spheroidal and bacillary lilac-colored metachromatic granules, most probably proteid in chemical nature. Such frequently collect in small groups and may thus simulate platelets. The resemblance is especially close where several such granules have collected within one of the minute oval coagula above described. It is only when constant reference is made to the platelets of the control blood-preparations that precise differences are established. In this work, whenever such a platelet-like body was encountered, reference was immediately made to the blood smears. The following are the essential criteria finally established for definitely distinguishing between such platelet-simulacra in lymph and genuine platelets of blood: The blood-platelets, like their simulacra in lymph, vary greatly in size; the size extremes of platelets are in the proportion of at least 1 to 10 volumes; but true platelets are very frequently collected into groups; their lymph simulacra are generally scattered and never in large groups. The peripheral hyaline layer of blood-platelets is very pale blue, the central granules violet, after Wright's stain; in the lymph simulacra the corresponding parts are pinkish-blue or light lilac, with deep lilac or violet granules. Moreover, the granules in the case of the lymph structures are coarser and less regular (spheroidal and bacillary) than those of true platelets.

The inference may perhaps be justly drawn on the basis of these comparative observations on lymph and blood, with respect to platelets, that the so-called 'platelets' of peptone and oxalate plasmas, on which is founded the precipitate-explanation of platelets (Buckmaster; Starling), are homologous with the platelet-simulacra of lymph. The evidence seems conclusive that

platelets are preformed structural elements of blood - not precipitated products—and that they do not form a normal constituent of lymph of the thoracic duct. This may mean only that the possibly small quota contributed to the peripheral portion of the lymphatic tree in relation to red bone-marrow, from which the relatively smaller amount of prothrombin and thromboplastic substance of lymph may have a partial source, suffered disintegration during their relatively slow progress towards the thoracic duct, and accordingly do consequently not appear as structural elements among the blood-cell content of thoracic-duct lymph.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, AUGUST 15

A DIFFERENTIAL INJECTION MASS FOR USE WITH STEREOROENTGENOGRAMS

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In studying the distribution of the blood-vessels within the lung and their relation to the bronchial tree, there are certain disadvantages connected with wax corrosions, celloidin corrosions and the clearing method of Spalteholz which can be overcome by special injection masses used in connection with stereoroentgenograms.

Wax corrosions are very brittle and easily broken; moreover, they do not show clearly the relation of the blood-vessels and bronchi to any variation in the lobation of the lung.

Celloidin corrosions are less brittle than wax corrosions and, when once they are mounted in their proper preservative, they are more permanent; but they also fail to retain the relationship of blood-vessels and bronchi to variations in lobation.

Spalteholz's method shows the lobation and its variations perfectly, but the blood-vessels and bronchi often form a confusing mass.

To offset these disadvantages, I have made use of the following method which shows variations in lobation, the distribution of the main arterial and venous trunks, and their relation to the bronchial tree.

The pulmonary artery is first injected with a starch mass in which vermillon granules are held in suspension; the pulmonary veins are next injected with a starch mass in which ultramarine-blue granules are held in suspension. When the injection masses have set the lungs are distended with air through a cannula and tube which is tightly tied in the trachea and a stereoroentgenogram taken. When this is viewed in the stereoscope it will be

found that the mass in the artery gives a uniform dense shadow; the mass in the vein gives a finely granular and less dense shadow; the bronchi distended with air have their walls well defined.

The use of starch masses for x-ray photographs is not new; but used in the manner I have indicated, by which a differential density is obtained, it is, so far as I know, new. The injection mass is prepared by mixing cornstarch and the pigment with 70 per cent alcohol until the required consistancy is obtained. If only the large vessels be desired, a mass that will flow with a pressure of 100 mm. of Hg. will give the desired results; but if the finer vessels are to be injected, the mass must be diluted with 70 per cent alcohol and the pressure increased.

No set rule can be given for the preparation of the injection masses, for I have found that no two lots give just the same results. Each new purchase requires one or two experimental trials before the best results are obtained. This is especially true of ultramarine blue, and in preparing an injection mass with this pigment a smaller quantity should be used than is the case with vermilion.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE

SURFACE VIEW OF INJECTED INTESTINAL VILLI

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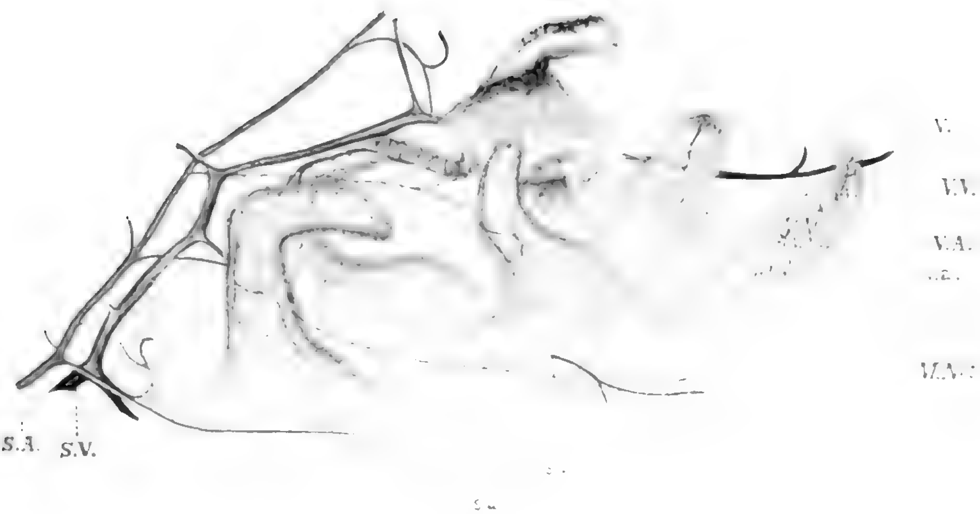
ONE FIGURE

The figure is from a small portion of a class microscopical demonstration which the writer prepared to illustrate the blood-vascular supply of a rabbit's intestine. It also shows much more conclusively than microscopical sections how the absorption surface of the intestine is enormously increased through the mucosa's being thrown up into numerous villi. When this preparation is examined with a binocular microscope the villi appear like numerous mountain peaks arising from the floor of the intestine.

This demonstration was prepared by first removing the blood from the intestine of a living rabbit by forcing a normal salt solution through the blood-vessels from the mesenteric vein. This was followed by an injection of chrome-yellow gelatin injecting mass, prepared after a formula given in a previous paper (Proc. Wash. Acad. Sci., 1905), which easily passes through the most minute capillaries. This injection was followed by a second injection of a carmine injection mass (formula given in above-mentioned paper) thickened sufficiently with cornstarch to prevent its passing through the capillaries of the villi. When this injection was completed the various mesenteric vessels were ligated and a portion of the intestine was removed and placed in cold water to solidify the injection masses. From whence it was transferred to a 10 per cent solution of formalin to fix. Small pieces of the intestine were then dehydrated, cleared in cedar oil, and mounted on a slide with dammar so that their inner walls and villi faced the cover-glass.

The writer takes pleasure in expressing his acknowledgments to one of his students, Mr. R. G. Young, for his skill in reproducing a small portion of this preparation in a drawing for the accompanying illustration (fig. 1).

It will be seen from figure 1 that two moderately large submucosa vessels (*S.A.* and *S.V.*) supply the immediate neighborhood with numerous branches (*s.a.* and *s.v.*). These vessels are likewise confined to the submucosa, but supply each villus with an arterial and a venous branch (*V.A.* and *V.V.*), which follow up opposite sides of a villus to its apex, within the connective-tissue layer close to the epithelium. In this preparation the villi arteries are fully as superficial as the corresponding veins. Each villus artery and vein sends off numerous branches to either side, which break up immediately into a capillary network in the tunica propria close to the basement membrane. This capillary network is considerably finer at the apex of a villus than at its base. Sometimes a number of these capillaries form a continuous vessel (*v.a.*) which follows the long axis of a villus close to the basement membrane. It should not be confused with the main villus artery or vein.



EXPLANATION OF FIGURE

The figure is from a drawing of a cleared microscopical preparation of a small portion of a rabbit's intestine, which had its arteries and capillaries filled with a yellow injecting mass and its veins with a red mass, looking at the inner surface. If any criticism is to be made of the drawing it is that the villi are a little too far apart. The arteries are colored red and the veins blue.

M.Net., mucosa capillary network	*s.v.*, branch of submucosa vein
S.A., submucosa artery	*V.A.*, Villus artery
s.a., branch of submucosa artery	*v.a.*, small villus vessel
S.V., submucosa vein	*V. V.*, villus vein

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE

AN INEXPENSIVE MICROSCOPIC PROJECTION AND DRAWING APPARATUS

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ONE FIGURE

This apparatus, as shown by the figure, consists of the Bausch & Lomb "Simple drawing equipment" supported by a stand and a second stand holding an adjustable drawing-board. The outfit serves every requirement for rapid and efficient work, notwithstanding its total cost, exclusive of the microscope, was about forty dollars.

The stand for supporting Bausch & Lomb's "Simple drawing equipment" has a wooden top (*A*) 22 x 14 inches, about 4 feet 3 inches from the floor and is supported by four legs constructed out of old gas pipe. These were bent outward at the base and strengthened 16 inches up by cross pieces. On top of the stand will be seen the Bausch & Lomb "Simple drawing equipment," which, including prism in front of the eyepiece for the microscope, is listed in catalogue at $32.50. As shown by the figure, the rheostat (*R*) is placed at the extreme left, next, in center, is a small arc light and condenser (*L*), elevated 4 inches by a wooden stool. At the extreme right a microscope is clamped on the adjustable microscopical stage and bent at right angles so the prism (*P*) extends over the center of the drawing-board. It is an easy matter to elevate or lower the microscope by the adjustable microscopical stand so that the condenser of the microscope (*C*) is in direct line with the light condenser (*L*.).

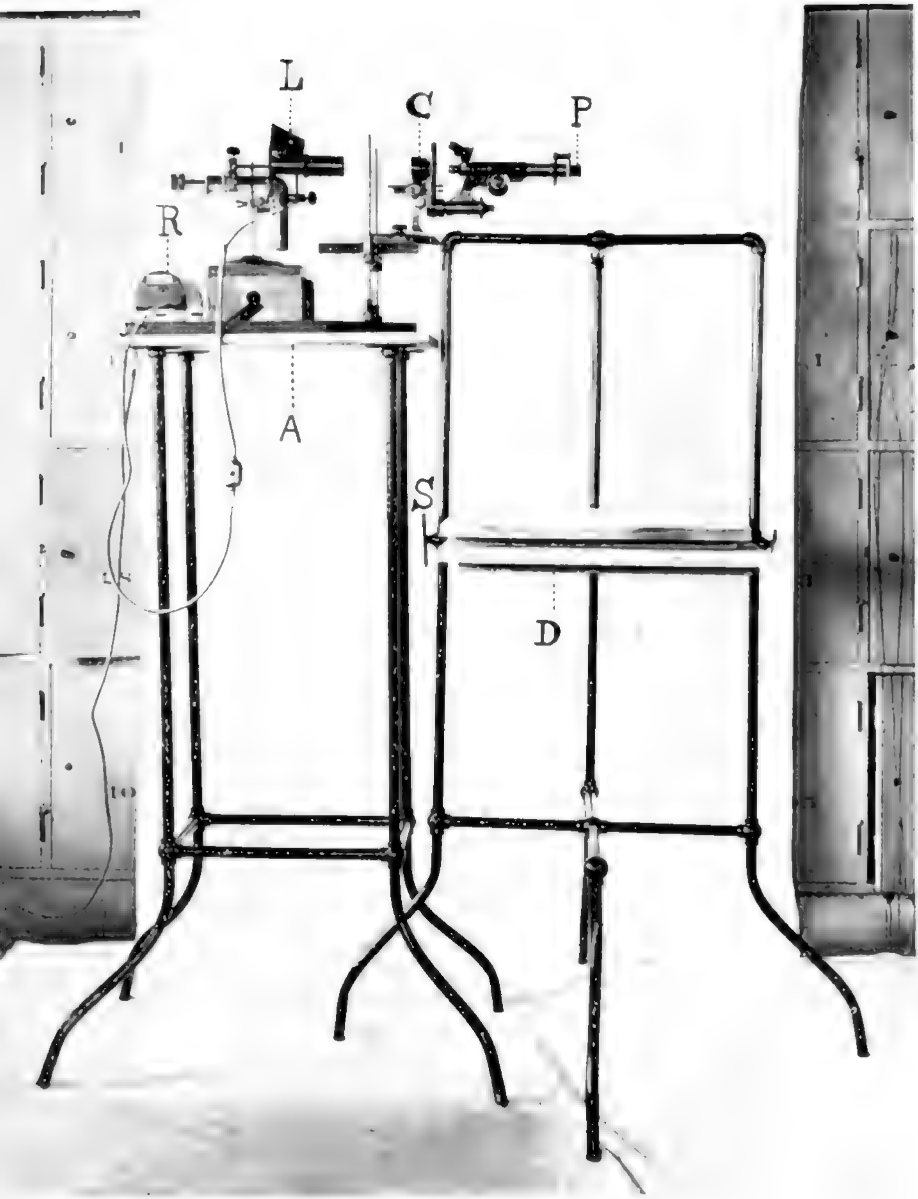
The framework for the adjustable drawing stand was also constructed out of old gas pipe. Its four legs were bent outward at base and about 6 inches from the floor were bound together

by cross pieces of the same material. As will be seen from the figure, uprights 3 feet 10 inches were screwed into the rear and two side legs and were connected at the top by cross pieces to give solidity. To provide ample room for drawing and centering the image on the drawing-board the two side legs and their uprights were placed 2 inches nearer the hind leg than the front leg. The drawing board (*D*) is of 1 inch cedar, 26 x 21 inches, and is attached to each of the three uprights by a metal clamp (*S*), which allows for clamping the drawing-board firmly at any desired distance from the eyepiece prism (*P*); so that the size of the image can be regulated from an inch in diameter to the total width of the drawing-board. The total amount of movement upward or downward of the drawing-board is 3 feet 10 inches and the adjustable microscopical stand can be raised about 6 inches, giving a total distance of about 4 feet 4 inches for projection of the image.

If a greater diameter of the image is desired, the adjustable drawing stand and the prism (*P*) may be discarded and the image can be projected on a vertical surface for class use or drawing a chart. For class demonstration the image can be projected on ground glass to advantage.

For low magnifications it is best to remove the microscope condenser (*C*) and depend entirely on the lantern condenser, or a third condenser placed before the microscope condenser will bring about the same results.

Fig. 1 is from a photograph of the microscopical projecting and drawing apparatus. *A*, stand for holding Bausch & Lomb's simple drawing equipment, *C*, microscope condenser, *D*, adjustable drawing-board, *L*, small arc light and condenser; *P*, prism over eyepiece; *R*, rheostat; *S*, clamp for holding adjustable drawing-board.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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, SEPTEMBER 16

A DUPLICATION OR BRANCHING OF THE NEURAL CANAL

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TWELVE FIGURES

In a previous article[1] concerning the lesions produced by electricity as observed after legal electrocution, the writer called attention to a duplication of the neural canal in one of the cases studied (Case III, G. G.).

This structural peculiarity was first noted in examining sections at the level of the motor decussation, and upon careful study it was found at the level of the sensor decussation also. The remaining unstained sections also were examined, and those that would give any assistance in the interpretation of this condition were stained and mounted. Of these the best and most characteristic were photographed. The accompanying illustrations represent only the canal area, and they have been so arranged that the top represents dorsal and the bottom ventral directions. Right and left represent the same respective directions as far as can be determined.

The order in which the illustrations have been placed seems to be the proper one according to the characteristics of the rest of each section. The first eight illustrations represent this peculiarity at the motor decussation level and the last four illustrations represent the condition at the sensor decussation level. The order of the sequence here may seem odd, perhaps, but an examination of the superficial arcuate nucleus and fibers materially assists in the arrangement, as progressive enlargement of these structures is readily noted. Throughout all of the sections the canal is much larger than seems usual and the direction of the long axis varies at the two levels here described.

[1] American Journal of the Medical Sciences, September, 1912

The first eight sections were photographed with a 16-mm. objective and a 10 × ocular, making a magnification of about 125 times. In the remaining sections the canal was larger so that the 7.5 × ocular was used with the 16-mm. objective giving a magnification of about 90 times.

Figure 1. In this section the canal shows a peculiar bulge, but the ependymal lining is distinct and unbroken throughout, though cilia are not demonstrable. The long axis of the canal is transversely placed and the immediate neuroglia is rather dense and devoid of nuclei. This seems to be the lowest section of the series.

Figure 2. In this section the canal is more extensive, laterally, than in figure 1, but less so dorsoventrally. The ependymal cells are clear and distinct, but cilia are absent. The neuroglia in the immediate area of the canal is dense and contains very few nuclei.

It is difficult to say which section comes next. If it is figure 3, then the canal has branched and the branches are at about their greatest distance apart and are about to approach each other. If figure 4 comes first, then the left canal (*l*) represents the direct continuation of the canal of the spinal cord while the right one (*r*) represents a blind branch.

Figure 3. This is the section in which the double condition was first noticed. Here are two distinct canals, the larger of which measures about 112μ by 63μ and the smaller 63μ by 43μ. They are about 224 microns apart. The ependymal cells are clear and distinct, but devoid of cilia. In the neuroglia immediately surrounding each canal there are many nuclei, while in the central part of the area between them the neuroglia seems quite dense.

Fig. 1 The canal of the oblongata at lower portion of the motor decussation level.

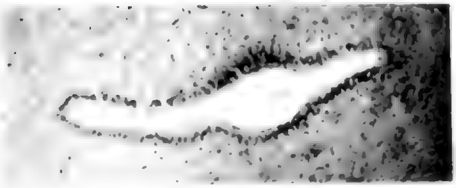
Fig. 2. The canal of the oblongata at a slightly higher level.

Fig. 3 The duplication of the canal. L, left; R, right.

Fig. 4 The two canals closer to each other.

Fig. 5 The curved canal. L, left limb; R, right limb.

Fig. 6 The canal at a higher level (reverse picture) L, left limb; R, right limb; *a*, branch from left limb.



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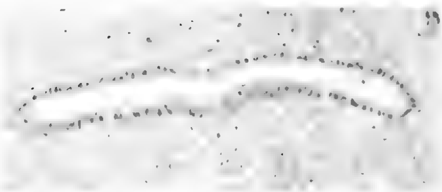

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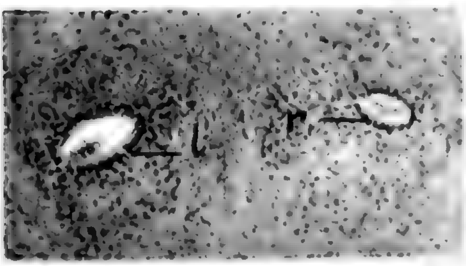

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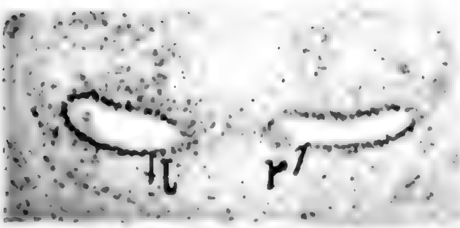

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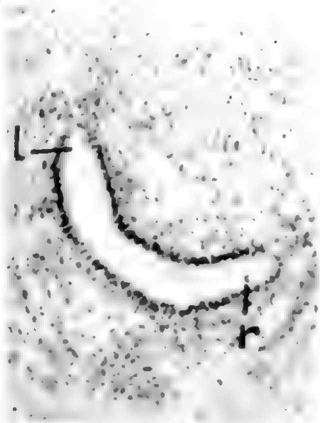

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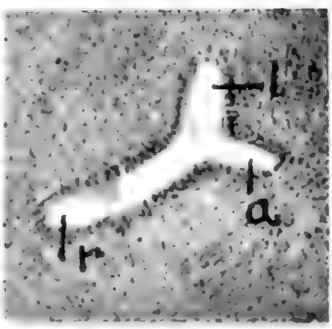

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Figure 4. In this section the two canals are rather large and close together. The ependymal cells are clear, distinct, and complete everywhere. The nuclear zone around each canal is not so well marked. If this section really precedes figure 3, then the canals are really diverging and one will end blindly. The writer, however, believes that they are converging and form the peculiar canal seen in figure 5.

Figure 5. In this section the canal is bent and the ependymal is clear except at the top of the left limb; here it shows a tendency to send cells into the glial tissue as though an extension of the canal were indicated. This becomes definite in higher sections.

Figure 6. This illustration is a reverse picture, so that the parts have been labeled as they should be and not as they appear. At this level the left limb (*l*) shows a tendency to extend the canal dorsally (as this limb points due dorsally). The right limb is unchanged. From the left limb, however, a branch (*a*) has developed and is quite extensive. The ependymal cells are clear and distinct throughout except in *l*, where an extension of the canal is forming.

Figure 7. In this section the right limb of the canal is still unchanged. The branch (*a*) is less distinct and it appears as though it will soon be closed off. The left limb shows a well-developed extension of cells at *b*; this outgrowth is almost as extensive as the canal. These cells seem to represent a bud that will become hollow and constitute another branch.

Fig. 7 The canal near the upper part of the motor decussation level. L, left limb; R, right limb; *a*, branch; *b*, cell cord extension from left limb.

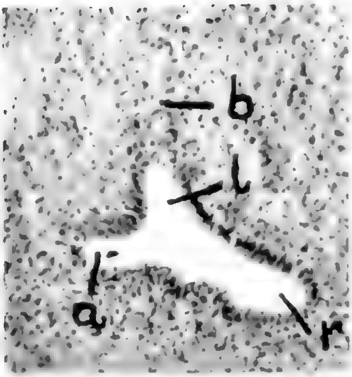
Fig. 8 The canal at the upper part of the motor decussation level. L, left limb; R, right limb; *a*, branch tending to occlusion; *b*, accessory canal.

Fig. 9 The oblongatal canal at the lower part of the sensor decussation level. D, dorsal; V, ventral; *a*, dorsal extension of ependymal cells.

Fig. 10 The canal at a slightly higher level. D, dorsal; V, ventral *a*, accessory canal; *b*, f-shaped group of connecting cells.

Fig. 11 The canal at a slightly higher level. D, dorsal; V, ventral; *a*, accessory canal; *b*, f-shaped group of connecting cells.

Fig. 12 The oblongatal canal at the upper part of the sensor decussation level. D, dorsal; V, ventral; *a*, accessory canal.



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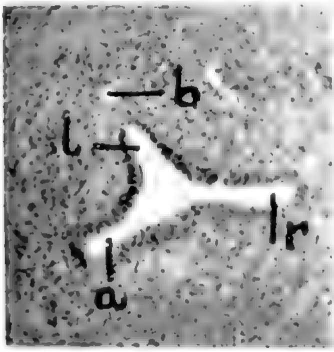

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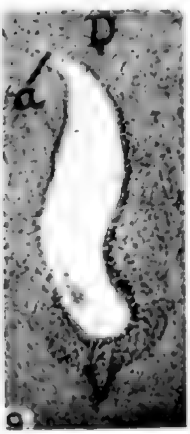

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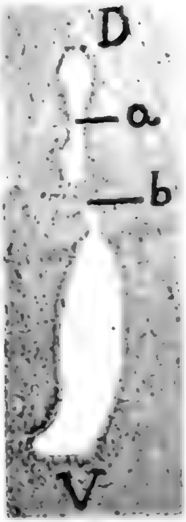

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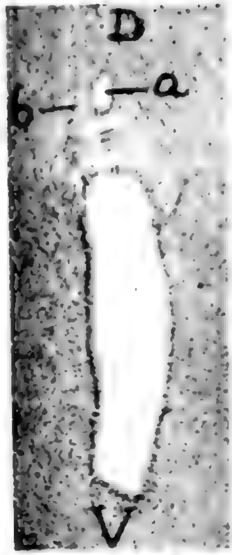

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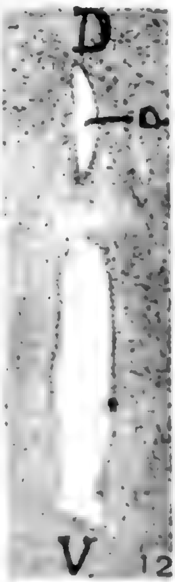

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Figure 8. In this section the right limb is as usual. The branch *a* seems to be closing. The left limb (*l*) has again distinct boundaries, but a part has been separated at *b*. This represents the budded cells of the preceding illustration, and these have now formed a little canal; in all probability this is a little blind diverticulum. Whether the branch *b* is a blind canal or joins the main canal is impossible to say, as no other sections at higher levels show it at all.

The succeeding sections are all at the sensor decussation level, and although the arrangement may seem odd it conforms to the internal structure of the oblongata at this level.

Figure 9. In this section the neural canal is again single. It is much larger than in the preceding sections even though the magnification is only 90 times. Its long axis is dorsoventrally directed and is peculiarly bent. The ependymal cells are clear and distinct except at the dorsal tip of the canal where they seem to be forming a dorsally directed bud.

Figure 10. In this section the canal is seen to be double. It seems as though the cells noted in the preceding section had formed quite a dorsal extension. These when hollowed out form another canal shown here at *a*. This canal is not completely free from the main canal, as at *b* there is the connecting group of cells. The ependymal cells of the main canal are distinct and clear except at the dorsal area where the accessory canal is connected.

Figure 11. This section shows a main canal still extensive; its ependymal is distinct except at the dorsal area. The accessory canal at *a* is much smaller than in the preceding illustration and its ependyma is not distinct. It would seem as though there was a tendency to occlusion. At *b* there is a ſ-shaped cord of cells that extends from the left side of the dorsal boundary of the main canal over and then down to the dorsal boundary of the accessory canal. These cells probably represent some of the general mass of budded cells from which the accessory canal is derived.

Figure 12. In this section the main canal is large and shows a tendency to branch at the lower right-hand corner. The

ependymal cells everywhere are clear and distinct. The accessory canal is larger than in the preceding section and it is well separated from the main canal. This accessory canal seems to be a blind diverticulum that arises at the lower part of the sensor decussation level and extends toward the mid-olivary level. It seems to be constricted and probably does not reunite with the main canal at all, but ends either blindly or by becoming part of the fourth ventricle as it (the branch) extends to higher levels.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, AUGUST 7

ON THE PROCESS OF DISAPPEARANCE OF THE CONUS ARTERIOSUS IN TELEOSTS[1]

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SIXTEEN FIGURES

Considerable difficulty has been encountered in the classification of fishes and their different genera, and especially in drawing a distinct line between the Ganoids and Teleosts.

Gegenbaur ('66) found in the Ganoids and in a limited number of Teleosts, intercalated between the ventricle of the heart and the truncus arteriosus, two segments clearly distinguishable from one another. Of these segments, the caudal is called the conus arteriosus or bulbus cordis, while the cranial is called the bulbus arteriosus.

The conus arteriosus is a well-marked muscular structure, furnished with numerous valves, and is one of the characteristics of the Elasmobranchs and Ganoids. Teleosts, with the exception of a very limited number, have no conus.

It was once thought that the rudimentary conus, or its total absence, with but one tier of valves, was characteristic of the Teleost heart. Stannius in his researches ('54) found that Albula vulpes possessed two tiers of valves instead of one. Likewise Senior ('07) found the same true of Megalops cyprinoides, Pterothrissus gissu, and Tarpon atlanticus. Amia calva has the shortest conus and a fewer number of valves than any other Ganoid. Some of the Teleosts seem to have descended from a stem somewhat akin to Amia, notably the herring group. Some of the families of this group, for instance, Albula vulpes, Pterothrissus gissu, Magalops cyprinöides, and Tarpon atlanticus have almost as large a conus as Amia itself, but with two rows

[1] Material used chiefly from the University and Bellevue Hospital Medical School collection.

of valves instead of three. Other nearly related genera have a distinct conus with only one row of valves.

Boas ('80), in his diagram of the Teleost heart, shows the valves to arise from fibrous tissue. This is erroneous, since it has since been found that all conus valves of Teleosts have their bases attached to muscle with some muscle fibers probably extending into the cusps. Boas' diagram for the Teleost represents the condition found in both Teleosts and Ganoids.

Favaro ('10) states that, from the point of view of comparative anatomy and embryology, we are not obliged to recognize in the conus and bulbus arteriosus autonomous and distinct organs, such as are observed in single orders and species, but rather structures which in part correspond to one another. Thereby he contradicts Gegenbaur's statement that the conus and bulbus arteriosus are separate and distinct segments. However, when one considers that Favaro had in mind only the part derived from vascular endothelium and Gegenbaur that derived from splanchnic mesoderm, both are correct. The intima and media of the bulbus are derived from embryonic vascular endothelium and are homologous with the intima of the conus and general vascular apparatus. Gegenbaur said that the conus differed from the bulbus in that the greater part of its thickness consists of heart muscle. This is quite true.

Hoyer ('00) advanced the theory that in Teleosts the conus had disappeared by intussusception into the ventricle.

I have examined serial sections of the hearts of the following fishes: Etrumeus teres, Coilia nasus, Clupea harengus, Ctengraulis mysticetes, Engraulis mordax, Clupanodon coeruleus, Stolephorus compressus, Harengula macropthalma, Chanos chanos, Opisthonema thrissa, Pomolobus pseudoharengus, Dorosoma cepedianum, Clupanodon lacepede, Notopterus, Pantodon, Mormyrus caballus, Alepocephalus agassizii, Osteoglossum bicirrhosum, Mesopus pretiosus, Thymallus signifer, Salmo irideus, Clupea alosa, Oncorhynchus chouicha, Osmerus mordax, Bathylagus benedicti, Notemigonus chrysoleucus, Elops saurus, Chrosomus erythrogaster, Chirocentrus dorab, Tarpon atlanticus, Hiodon tergisus, Campostoma anomalum, Albula

vulpes, Pterois volaus, Plotosus anguillaris, Cantherines sandwicheusis, Balistapus undulatus, Ostracion cornutum, Chaetodon aetifer, Chaetodon trifasciata, Heterotis niloticus, Ophiocephalus striatus, Hippocampus atterimus, Syngathus peleagicus Gastrotokens biaculeatus, Amia calva, Polyodon spathula, and Lepidosteus plastostomus.

The specimen of Heterotis niloticus and Osteoglossum bicirrhosum measured 40 cm. in length. The others ranged from 6 to 20 cm. The illustrations as here reproduced are not all of the same magnification. It was considered most convenient for comparison to employ any magnification necessary to distinctly show the conus, when present, and the attachment of its valves. The figures are arranged in the order of what seemed to be the progressive disappearance of the conus arteriosus as found in specimens here studied.

I take this opportunity to thank Professor Senior, of The University and Bellevue Hospital Medical College, for the privilege of using material in the collection of this institution, and I sincerely thank Professor Ruth, of the University of Manila, Professor Jordan, of Leland Stanford University, and Mr. Rathbun, of the United States National Museum, for their kindness in sending me a number of the specimens enumerated.

I find that the hearts of Tarpon atlanticus, Osteoglossum bicirrhosum, Ophiocephalus striatus, Chaetodon aetifer, Coilia nasus, Chanos chanos, Albula vulpes, Notopterus, Plotosus anguillaris, Dorosoma cepedianum, and Heterotis niloticus, all show a distinct muscular conus. Figure 1 illustrates such a conus in Dorosoma cepedianum and figure 2 the similar condition in Heterotis niloticus. It was deemed unnecessary to submit illustrations of the remaining specimens mentioned since the conus in them was of similar appearance and structure.

The hearts of Balistapus undulatus, Pterois volaus, Stolephorus compressus, Pomolobus pseudoharengus, Elops saurus, Clupanodon coeruleus, Harengula macrothalma, Opisthonema thrissa, Engraulis mordax, Alepocephalus agassizii, and Ctengraulis mysticetes show a rudimentary conus of elastic tissue including a small amount of scattered cardiac muscle. These are shown in figures 3, 4, 5, 6, 7, and 8, respectively.

EXPLANATION OF THE FIGURES

The figures are all drawn to include only the cephalic ends of the hearts of the specimens named. The sections from which the drawings were made were selected from serial sections of the hearts and, as is evident, many of the sections pass obliquely through the cephalic ends. The figures represent hearts of specimens as follows:

1 Dorosoma cepedianum
2 Heterotis niloticus
3 Clupanodon coerlueus
4 Harengula macropthalma

5 Ctengraulis mysticetes	11 Bathylagus benedicti
6 Engraulis mordax	12 Syngathus peleagicus
7 Opisthonema thrissa	13 Hiodon tergisus
8 Alepocephalus agassizii	14 Hippocampus atterinus
9 Mormyrus caballus	15 Chirocentrus dorab
10 Clupea harengus	16 Pantodon

REFERENCE LETTERS

B A, bulbus arteriosus C A, conus arteriosus. V, ventricle

In the remaining number of Teleosts examined, the conus seems to have disappeared. The greater number of them appear to have lost it by intussusception (telescoping) into the ventricle. In figure 9, Mormyrus caballus, the valves are seen to be attached to intussuscepted conus muscle, caudal to the cephalic end of the heart, while on the other hand, in some that have lost their conus, one finds the conus valves not to be drawn into the ventricle, as in figure 9 but to be attached to its most cephalic end, as in figure 10, Clupea harengus. In the hearts of Bathylagus benedicti, Syngathus peleagicus, and Hiodon tergisus, figures 11, 12, and 13, respectively, the cusps are solely bulbar in their attachment and show no evidence of being drawn into the ventricle nor any direct structural relation with it. In Hippocampus atterimus and Chirocentrus dorab the valves are merely attached to the bulbus and to the aortic end of the ventricle with also no evidence of being drawn into the latter, while in the heart of Pantodon both cusps are attached to the caudal end of the bulbus, which, on the left side, is drawn into the ventricle.

I believe that Hoyer's statement that in Teleosts the conus has been lost by intussusception or recession into the ventricle applies to most species of Teleosts, but, on the other hand, it seems to me that in those hearts one should find the conus valves caudal to the aortic end of the heart, with their bases attached to the receded conus muscle. In the figures showing no conus and no drawing in of the valves, and in those showing the valves to have only bulbar attachment with no intussusception, it appears to me that in a limited number of Teleosts the conus is not intussuscepted into the ventricle, but is taken up by the caudal elongation of the bulbus.

The phenomenon of the disappearance of the conus arteriosus without intussusception of the valve cusps into the ventricle, as noted, may possibly be explained physiologically. One may consider that, on ventricular contraction when the blood is forced into the conus and bulbus, if during the succeeding contraction of the bulbus, the greater resistance offered to the passage of the blood through the branchial vessels necessitates a

greater pressure and a hypertrophy of the bulbus, then there would result a stronger caudal regurgitation of the blood against the cusps of the conus valves. This caudal regurgitation might lead to a caudal compression and consequent obliteration of the conus.

I desire to express my appreciation to Professors H. D. Senior and Irving Hardesty for their aid and suggestions offered me in the preparation of this paper.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, AUGUST 7

THE DEVELOPMENT OF THE HYPOPHYSIS OF THE ANURA

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EIGHTEEN FIGURES

Recent studies have shown that the epithelial portion of the hypophysis consists of three parts. It has been clearly demonstrated that these three parts are distinct both ontogenetically and histologically. Besides the anterior lobe proper and the pars intermedia previously recognized, Tilney ('13) has shown that a third epithelial part, the 'pars tuberalis' is to be distinguished in mammals and in birds. He gives a brief account of its development in the cat and in the chick. Woerdeman ('14) treats of early stages in the development of an homologous part, the 'lobulus bifurcatus,' in reptiles, birds, and mammals. Baumgartner ('16) traces the development of the 'pars tuberalis' in reptiles. Parker ('17) has described its ontogeny in the Marsupialia. In a recent paper ('18) the writer has given a detailed account of the development of the hypophysis of the rabbit and has followed the differentiation of the three epithelial parts until the time of birth.

The most striking feature in the development of the pars tuberalis is its paired origin. This is noted by all of the authors above enumerated. In the rabbit the pars tuberalis is discernible very early. From the thickened epithelium which lies in front of the early Rathke's pocket two thickened ridges are formed. These are the anlagen which fuse and form the 'pars tuberalis'—a thin lamina surrounding the infundibular neck and spreading out under the tuber cinereum.

The pars tuberalis is in many forms considerably more vascular than is the pars intermedia. It is further characterized by the tubular or acinar arrangement of its cells. Tilney

states that the walls of these acini are composed of one or two layers of cells, while Parker and Atwell speak of them as being composed of one layer only.

Woerdeman ('14) draws an interesting homology between the pars tuberalis (which he terms the 'lobulus bifurcatus' following Bolk) and the inferior sacs of the Elasmobranch fishes, which, as is well known, have a paired origin. Woerdeman lacked material showing the development of the hypophysis in the higher fishes and in the Amphibia. On this account the writer felt that such an homology must be considered precarious until the ontogeny of the hypophysis had been studied for these remaining vertebrate classes (Atwell, '18).

The present study was undertaken for the purpose of ascertaining whether a homologue of the pars tuberalis is to be recognized in the amphibian hypophysis. In answering this question it was hoped that light would be shed on the question as to whether a lobe comparable to the pars tuberalis is constantly present in the hypophysis of all vertebrates.

THE DEVELOPMENT OF THE HYPOPHYSIS IN THE ANURA

The material for this study consists of some eighty series of sections of larvae of Rana pipiens, which range in length from 2 to 25 mm. This was augmented by the preparation of series of larval Rana clamitans obtained just preceding and during metamorphosis. For the hypophysis of the adult frog, specimens of R. pipiens and of R. catesbiana were used. For purposes of comparison several series of larvae of a toad (Bufo americana) were also prepared.

A series of wax-plate reconstructions was made from typical larval and adult stages to illustrate the morphogenesis of the hypophysis. The hypophysis of the adult frog was first studied in the gross and sketched at a low magnification, using the camera lucida. After sectioning, the same structures were identified and studied under higher powers. Graphic reconstructions were made and compared with the sketches obtained in the gross.

It is not the purpose of this study to treat at length the early appearance of the hypophysis. This has been done for the Amphibia by Goette ('75), Orr ('89), Haller ('97), and Corning ('99). Of the large number of series of sections studied only certain typical stages have been selected for description here.

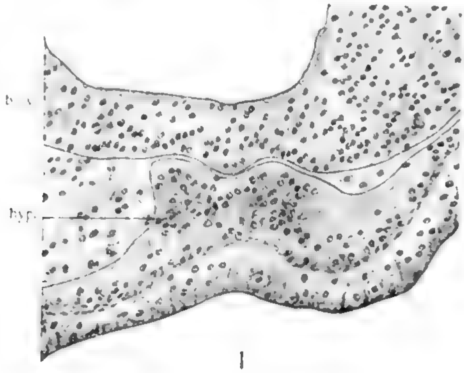
3-mm. larva of Rana pipiens. At this stage the anlage of the hypophysis is already well formed. The ectoderm at the anterior end of the embryo is separated into two well-defined layers. It is from the inner of these that the hypophysis arises. In sagittal sections the hypophysial anlage shows as a wedge-shaped mass of cells lying between the neural tube and the wall of the foregut. The apex of the wedge is directed caudally. There is usually considerable separation between the two layers of ectoderm at the base of the wedge. The appearance given is that an attempt had been made at evaginating to form an hypophysial pocket.

A transverse section from near the anterior end of the hypophysis is presented in figure 1. It is seen that this part of the anlage shows a distinct bilaterality. Reconstructions of the entire hypophysis at this stage show that this bilaterality is confined to the anterior portion of the gland. Possibilities as to the significance of these appearances are discussed on a later page.

7-mm. larva of R. pipiens. A sagittal section of a 7-mm. larva is shown in figure 3. The oral plate is intact. The hypophysis, *hyp.*, extends caudally from its attachment to the inner layer of the ectoderm. It does not reach to the caudal end of the thin-walled infundibulum, *inf.* A reconstruction, from sagittal sections (fig. 5) shows that the hypophysis maintains its attachment to the ectoderm by a relatively broad band. Just caudal to the stalk the hypophysis is widened and shows a pair of thinner lateral shelves. It is believed that these give rise to the more definite lateral lobes of later stages.

8-mm. larva of R. pipiens (fig. 6). The hypophysis has recently lost its attachment to the ectoderm. It lies flattened out under the floor of the infundibulum and is now somewhat wider from side to side than nasocaudally. Its caudal end is now almost

even with the caudal extremity of the infundibulum. The rather sudden breaking loose of the hypophysis from the ectoderm has resulted in a marked change in the shape of the gland. Before (fig. 5) it was much longer in a nasocaudal direction. Now (fig. 6)



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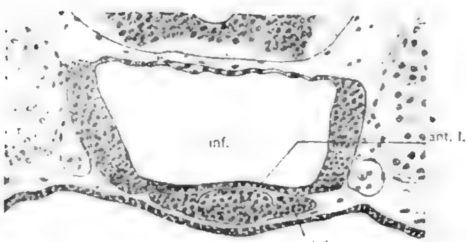

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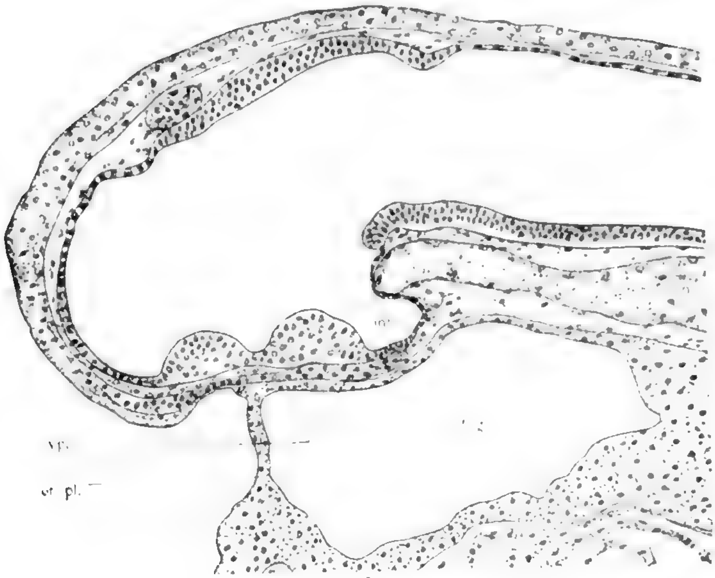
Fig. 1 Transverse section of anterior end of hypophysis of a 3-mm. larva of Rana pipiens, showing bilaterality of hypophysis anlage; *hyp.*, hypophysis; *b.w.*, brain wall. × 100.

Fig. 2 Transverse section of hypophysis region of a 12-mm. larva of R. pipiens. *l.l.*, lateral lobes; *ant. l.*, anterior lobe proper; *inf.*, infundibulum. × 100.

it is spread out from side to side. This rearrangement is a very disturbing factor in any attempt to follow the lateral lobes. They cannot be distinguished with certainty at this stage.

12-mm. larva of R. pipiens. A transverse section from an embryo of this length is shown in figure 2, a sagittal section in



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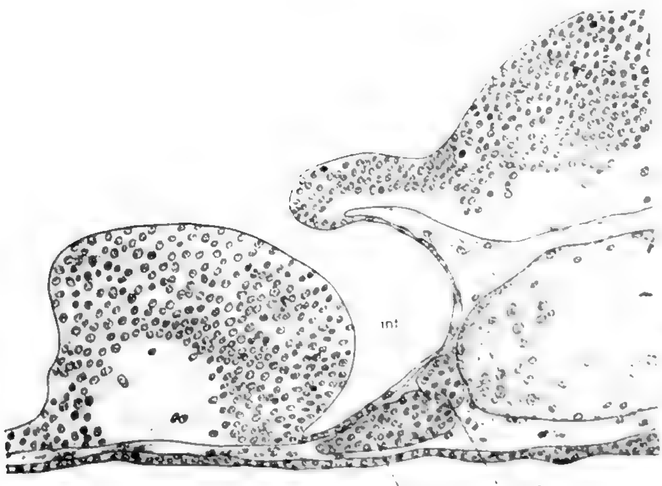

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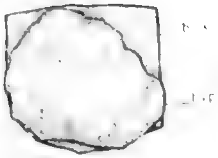
Fig. 3 Midsagittal section of head end of a 7-mm. larva of R. pipiens *n.c.*, notochord; *inf.*, infundibulum; *f.g.*, foregut; *hyp.*, hypophysis; *or. pl.*, oral plate. × 75.

Fig. 4 Midsagittal section of hypophysis region of a 12-mm. larva of R. pipiens. *ant. l.*, anterior lobe proper; *p. int.*, pars intermedia; *inf.*, infundibulum; *nc.*, notochord. × 150.



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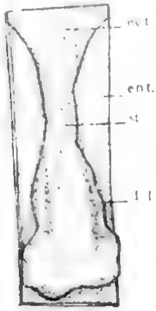

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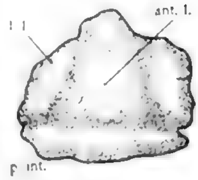

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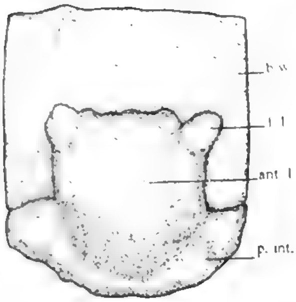

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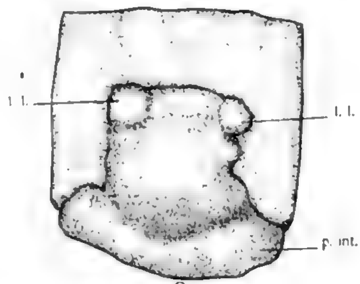

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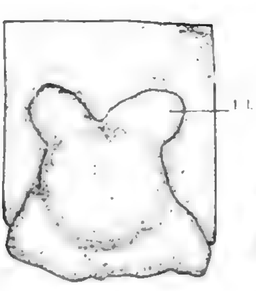

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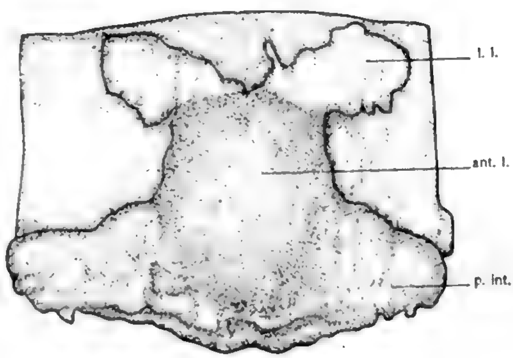

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Fig. 5 Reconstruction of hypophysis of a 7-mm. larva of R. pipens, viewed from the dorsal surface, caudal end at the bottom. *l.l.*, lateral lobe; *ent.*, entoderm; *ect.*, ectoderm; *st.*, epithelial stalk. × 100.

Fig. 6 Reconstruction of the hypophysis of an 8-mm. larva of R. pipiens, viewed from the ventral surface; caudal end is below. *hyp.*, hypophysis; *b.w.*, brain wall. × 100.

figure 4, and a dorsal view of a wax model of the epithelial hypophysis in figure 7. It will be seen that the hypophysis has its caudal end in close proximity to the anterior end of the notochord. The latter, in earlier stages, touched the dorsocaudal wall of the infundibulum, causing it to be indented (fig. 3). The hypophysis is already differentiated into three epithelial portions, one of which is paired. The anterior lobe proper is the thickened central portion of the gland. At its caudal end it is bounded by a bulging transverse ridge, the pars intermedia. From the sides a pair of thinner shelves or ledges (figs. 2 and 7) extend; these are the lateral lobes, the paired anlagen of the pars tuberalis.

18-mm. and 20-mm. larvae of R. pipiens. In these and all subsequent stages the morphological differentiation of the three epithelial portions is very distinct. In figures 8 and 9 models of these stages are viewed from the ventral surface, with the caudal end below. The anlagen of the pars tuberalis are visible as a pair of buds (*l.l.*) located at each side of the anterior end of the hypophysis. At the caudal end of the gland a curving transverse ridge, marked off by a groove, is the pars intermedia. This ridge is considerably longer than the width of the remainder of the gland.

A thickening at the caudal end of the infundibulum, corresponding to the extent of the pars intermedia, is the beginning of the neural lobe. It lies just dorsal to the pars intermedia and so is not visible in a ventral view of the reconstruction.

Fig. 7 Reconstruction of the epithelial hypophysis of a 12-mm. larva of R. pipiens, viewed from the dorsal surface. *l.l.*, lateral lobe; *p. int.*, pars intermedia; *ant. l.*, anterior lobe proper. × 100.

Fig. 8 Reconstruction of the hypophysis of an 18-mm. larva of R. pipiens, from ventral surface; caudal end below. *ant. l.*, anterior lobe proper; *p. int.*, pars intermedia; *l. l.*, lateral lobe; *b.w.*, brain wall. × 100.

Fig. 9 Model of hypophysis of a 20-mm. R. pipiens larva, from ventral surface; caudal end below. Abbreviations as for figure 8. × 100.

Fig. 10 Model of the hypophysis of a 22-mm. larva of R. pipiens, from the ventral surface. Caudal end below. Abbreviations as for figure 8. × 100.

Fig. 11 Model of the hypophysis of a 24-mm. larva of R. pipiens, from ventral surface. Abbreviations as for figure 8. × 100.

Already the two show evidences of coming into intimate relation with each other.

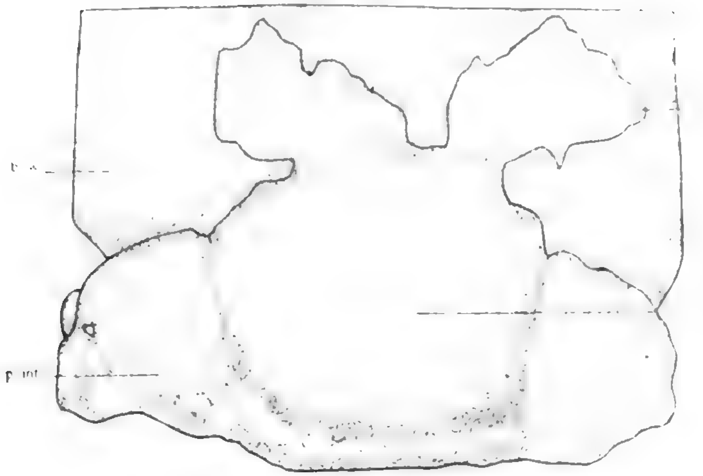
22-mm. and 24-mm. larvae of R. pipiens. Ventral views of wax-plate reconstructions made from these embryos are shown in figures 10 and 11. The lateral lobes, *l.l.*, which are the anlagen of the pars tuberalis, are larger and are flattened out under the infundibular floor. The pars intermedia conforms more closely to the shape of the neural lobe (fig. 11).

Larvae of R. clamitans and Bufo americana at metamorphosis. Very similar relations to the last are shown by a larva of R. clamitans possessing very short hind legs. A model from this embryo is shown in figure 12. That the spreading out of the lateral lobes under the infundibular floor is due to active growth is indicated by the irregularity in outline of the lateral lobes at these stages (figs. 11 and 12). Other larvae of R. clamitans obtained at later stages of metamorphosis show the lateral lobes in the process of separation from the anterior lobe.

A reconstruction from a toad (Bufo americana) which had just completed metamorphosis is presented in figure 13. The lateral lobes, *l.l.*, are seen to be joined. They are united to the anterior lobe proper by a single attenuated epithelial strand. The lateral lobes do not long remain united, however, for another toad of about the same age (not figured) shows one lobe entirely free, while the other is attached to the anterior lobe by a narrow strand.

From what has been observed in R. clamitans and B. americana it seems safe to assume that in general the lateral lobes become separated from the remainder of the epithelial hypophysis at the completion of metamorphosis or very soon thereafter.

Adults of R. pipiens and R. catesbiana. In the adult frog the pars tuberalis, derived from the two lateral lobes, is seen as a pair of epithelial plaques lying close under the 'infundibular' floor some distance in front of the remainder of the gland (figs. 14 to 18). Each plaque is approximately circular, but not infrequently shows a greater diameter from side to side. Each is 0.3 to 0.4 mm. in diameter and about one-fifth as thick. They



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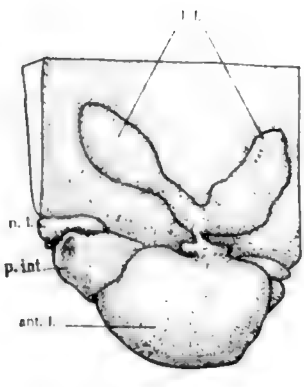

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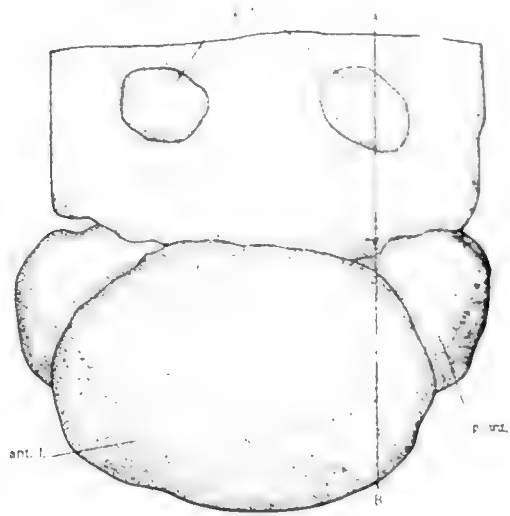

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Fig. 12 Ventral view of a model of the hypophysis of a larva of R. clamitans with small hind legs (legs 6 to 7 mm. long). *ant. l.*, anterior lobe proper; *p. int.*, pars intermedia; *l.l.*, lateral lobe; *b.w.*, brain wall. × 100.

Fig. 13 Ventral view of a model of the hypophysis of a toad (Bufo americana) which had just completed metamorphosis. *n. l.*, neural lobe; other abbreviations as for figure 12. × 100.

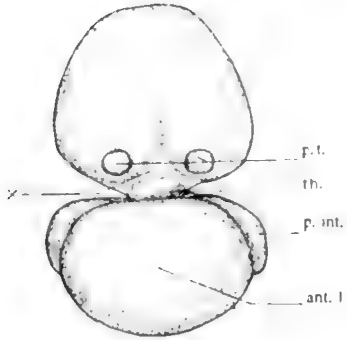
Fig. 14 Model of the hypophysis of an adult of R. pipiens, viewed from the ventral surface. *p.t.*, pars tuberalis; other abbreviations as for figure 12. *A-B*, plane of section shown in figure 18. × 35.

lie close to the brain wall, indenting but not invading it, figure 18, and are surrounded by the pia mater.

By a proper combination of dehydrating and clearing fluids the pars tuberalis may be seen in the gross, preferably with the aid of a binocular microscope or a low-power dissecting lens. Using a mixture of absolute alcohol, 60 parts, and xylol, 40 parts, it was possible to obtain the camera-lucida sketch shown in figure 15, which reproduces the ventral surface of the hypophysis of an adult bullfrog (R. catesbiana). Similarly the drawing from an adult specimen of R. pipiens presented in figure 16 A was obtained. To make sure that the parts observed in the gross were the same as those seen in sections, these two brains were later cut into transverse series. A graphic reconstruction of the caudal half of the so-called infundibulum and adjacent structures of one is shown in figure 16 B, and a single transverse section passing through the center of the pars tuberalis is presented in figure 17.

It will be seen that the two plaques composing the pars tuberalis lie under fairly thick lateral portions of the 'infundibular' floor. Between these thickenings and caudal to them the floor is very thin, X, Fig. 15. When observed in the gross this thin floor is almost transparent and this fact, no doubt, has given rise to the rather misleading description of the infundibulum as a "bilobed structure, emarginate posteriorly and divided by a median longitudinal groove" (Holmes, p. 294). Such relations are not evident from a ventral view of a reconstruction (Fig. 14). It seems to me doubtful whether the thickened 'bilobed' portion is properly to be considered as a part of the true infundibulum. I would restrict the name infundibulum to that portion possessing the very thin floor, X, figure 15, and exhibiting a single medial thickening at its caudal end, *th.*, figure 15 (see also fig. 16). Possibly the bilateral thickenings, from their more anterior position, are to be considered homologous with the tuber cinereum of the higher vertebrates.

Another feature of interest is the presence of small but well-marked depressions, one above each half of the pars tuberalis, figure 17. Whether these depressions are to be considered



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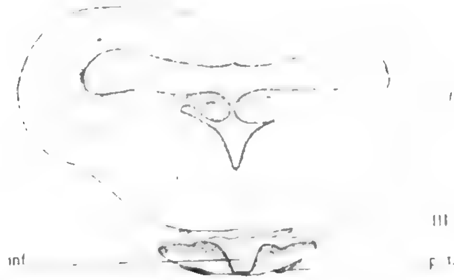

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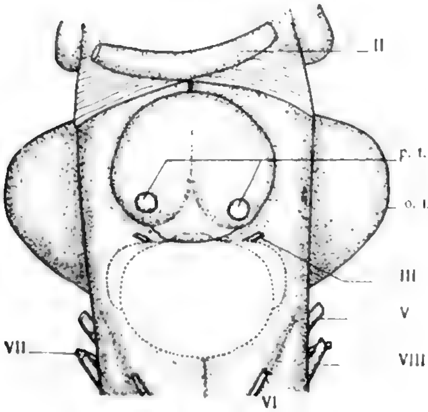

A

B

16

Fig. 15 Camera-lucida drawing of the 'infundibulum' and hypophysis of an adult of R. catesbiana, from the ventral surface. Caudal end is below. *ant. l.*, anterior lobe; *p. int.*, pars intermedia; *p.t.*, pars tuberalis; *th.*, thickening of infundibular floor; *X*, thin portion of infundibular floor. × 10.

Fig. 16 A Camera-lucida drawing showing a portion of the ventral surface of the brain of an adult R. pipiens. Main body of hypophysis had been removed; its approximate position is indicated in dotted outline. *p.t.*, pars tuberalis; *o.l.*, optic lobe; *II*, *III*, *V*, *VI*, *VII*, *VIII*, cranial nerves. × 10.

B Graphic reconstruction, from transverse sections, of portion adjacent in 'A'.

Fig. 17 Transverse section of the frog's brain shown in figure 16 A. *p.t.*, pars tuberalis; *III*, oculomotor nerve; *o.l.*, optic lobe; *inf.*, infundibulum. × 12.

homologous with the 'tuberal recesses' of higher vertebrates is uncertain.

The anterior lobe proper has become the most ventrocaudal portion of the gland. This lobe, then, does not deserve the name 'anterior' from its adult position in the frog, but from its very evident relation to the homologous lobe possessed by higher vertebrates. The lobe is oval in outline, being shorter in its nasocaudal dimension.

The pars intermedia is a long transverse ridge with bulging lateral terminations. It conforms in extent to the neural lobe which is definitely constricted from the infundibulum dorsally and at the sides. In handling, the hypophysis readily separates from the infundibulum, the break occurring at the narrow attachment of the neural lobe (fig. 18). In such a case the neural lobe, pars intermedia, and anterior lobe proper come loose as a unit. The pars tuberalis remains attached to the floor of the part interpreted as the tuber cinereum (fig. 16A).

In sagittal sections the neural lobe is cut in a direction transverse to its greatest length (fig. 18). As Stendell ('14) has noted, it presents in this section the form of a right-angled triangle with its hypotenuse lying against the pars intermedia.

The three epithelial lobes are histologically distinct. The anterior lobe proper, which has been so named from its resemblance to the anterior lobe of higher vertebrates, is very vascular and consists of cords of cells which are chromophilic and granular. The pars intermedia and the pars tuberalis may both be spoken of as chromophobic portions of the gland. There are, however, well-defined differences between the two. The pars intermedia of the frog, in contrast to the same lobe in mammals, for example, is traversed by numerous blood-vessels. The pars tuberalis, on the other hand, and this is also in contrast to the mammalian gland, is not invaded by vessels at all. This may in part be accounted for by its small size in the frog. Many of the cells of the pars intermedia (adult R. catesbiana) contain rounded hyalin bodies, considerably smaller than the nucleus. These seem to be more numerous in the neighborhood of the blood-vessels. Such hyalin bodies are not to be found in the pars tuberalis.

DISCUSSION

Entoderm. My observations bear out the assertion of Corning ('99) that entoderm does not enter into the development of the frog's hypophysis after the manner described by Kupffer ('94) and Valenti ('95). There is indeed a mass of cells, epithelial in appearance, in relation with the anterior end of the notochord in larvae of R. pipiens having a length of 2.5 to 3 mm. I am not able to find evidence that this mass becomes incorporated into the hypophysial anlage. That this epithelium-like mass is comparable with the 'protochordal plate' of other vertebrates, as suggested by Corning, Mrs. Gage, and more recently by Parker, seems to me not unlikely.

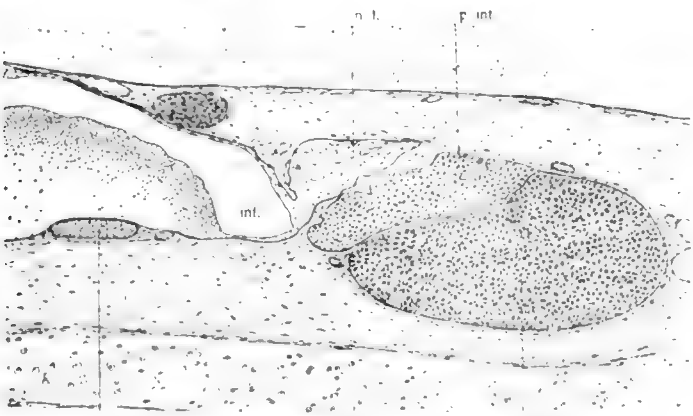

Fig. 18 Paramedian sagittal section of hypophysis of an adult frog (R. pipiens). Plane of section indicated by line A-B, figure 14. Nasal end is at the left. *inf.*, infundibulum; *p.t.*, pars tuberalis; *n.l.*, neural lobe; *p. int.*, pars intermedia; *ant.*, *l.*, anterior lobe proper. × 50.

The pars tuberalis. A two-fold origin for the hypophysis of Ambylstoma has been recorded by Kingsley and Thyng. These observers believe that the bilaterality involves the entire gland and that the two parts soon fuse. The bilaterality observed in the frog's hypophysis seems, in certain stages at least, to be confined to the anterior portion of the anlage and does not extend to its caudal tip. I have never seen convincing evidence that the two parts come together and fuse in the frog.

It is with some difficulty that the two parts may be traced to the bud-like lateral lobes which later form the pars tuberalis.

When the hypophysis anlage breaks loose from its parent ectoderm, there is a very noticeable rearrangement in its shape, as has been noted. This is a disturbing factor in any attempt to trace the history of the lateral lobes. During the stages just preceding and following the separation, however, a somewhat thinner shelf is seen on each side of the anterior half of the anlage (figs. 5 and 7). These I have supposed to be the lateral lobes.

By the time the embryo has attained a length of 18 mm. the lateral lobes are definite bud-like structures at each side of the nasal end of the gland (fig. 8). The further development of the pars tuberalis consists in a spreading out of the lateral lobes under the brain floor and their subsequent detachment from the anterior lobe proper. This latter occurs, in Rana clamitans and Bufo americana at least, during the latter part of metamorphosis or very soon after its completion.

In the adult frog the pars tuberalis consists of a pair of epithelial plates, free from each other and from the remainder of the gland. The plates have been described by a few authors, but all have spoken of them as insignificant rudiments and none has considered them homologous with the lateral lobes seen during the development of the hypophysis of higher vertebrates.

The terminology for the various lobes of the hypophysis differs so much among writers that it is customary to gather into a table the names to be found in the literature. In the following table I have included only those authors who speak of three lobes of the Anuran hypophysis.

Terminology of the lobes of the Anuran hypophysis

OBSERVER	ANTERIOR LOBE PROPER	PARS INTERMEDIA	PARS TUBERALIS
Gaupp ('89)	Pars posterior	Pars anterior	Partes laterales
B. Haller ('97)	Hypophysis	Infundibular-drüse (Saccus-vasculosus)	Vorderlappen-paares
Stendell ('14)	Haupt Lappen	Zwischen Lappen	Pars anterior or Pars chiasmatica
Atwell ('18)	Anterior lobe proper	Pars intermedia	Pars tuberalis

GENERAL CRITERIA FOR HOMOLOGIZING THE LOBES OF THE HYPOPHYSIS IN THE VARIOUS VERTEBRATE CLASSES

To homologize the lobes of the hypophysis throughout the vertebrate classes it is necessary to have clearly in mind both their developmental and their adult relations. I propose to enumerate some of the criteria by which they may be classified in the light of some of our recently acquired knowledge. It must be borne in mind that many of the points to be given are as yet supported by relatively few observations. However, it is hoped that their presentation in concise form may be found useful. The adult relations may first be considered.

Size. In general the anterior lobe proper is the largest of the three lobes, the pars intermedia the next smaller and the pars tuberalis the smallest. That these relations may not hold constant in all vertebrates is quite possible, but they are certainly true for the frog and for some mammals. Woerdeman ('14) believes that the pars tuberalis (his 'lobulus bifurcatus') becomes progressively less important as the vertebrate scale is ascended.

Form and position. The anterior lobe proper tends to maintain an approximately spherical or ovoid shape. It is not moulded to any marked degree by surrounding structures. It is not intimately attached to any tissue of nervous origin and in many forms does not even lie in contact with neural tissue. On this account Tilney ('13) has named this lobe the 'pars distalis.'

The pars intermedia is always conformed to the shape and extent of the neural lobe. In reptiles, birds, and mammals the pars intermedia is a thin epithelial layer applied to the neural lobe and derived from the superodorsal wall of Rathke's pocket. It more or less completely surrounds the neural lobe, which in these forms has its longest dimension in the sagittal direction. Later in life the pars intermedia invades the tissue of the neural lobe to a considerable extent. In the frog also the pars intermedia corresponds to the neural lobe in shape. Here the latter has its long axis extending from side to side. The pars intermedia has a very similar shape, with the exception that it is

rounded and bulging where it protrudes beyond each side of the anterior lobe (figs. 14, 15, and 16).

The pars tuberalis likewise conforms to the shape of that part of the brain wall upon which it lies. It is essentially thin and lamina-like. In mammals it surrounds the infundibulum and spreads out under the tuber cinereum. Reconstructed and viewed separately, it may be described as 'saucer-shaped' (Tilney, '13) with a perforation for the infundibular neck. In reptiles thin bands and zones may be formed about the middle of the anterior lobe in addition to the thin plate which is spread out under the brain floor (Baumgartner, '16). In the Anura the pars tuberalis does not extend dorsal or caudal to the attachment of the neural lobe. It consists merely of a pair of small rounded plaques, located nasalward from the remainder of the gland.

It is important to note the relations of the pars tuberalis to the membranes of the brain. It lies in the pia mater in close relation to the brain floor, but does not appear to invade the neural tissue after the manner so characteristic for the pars intermedia.

Histology. Much remains to be worked out in regard to the normal histology of the hypophysis. It is my purpose merely to emphasize the fact that the three lobes may be readily distinguished by their histological characters. Tilney ('13) was first in attempting to classify the differences in structure of the three parts. This he did for a number of mammals and for the domestic fowl. According to Tilney, the three parts may be differentiated as to cell structure and vascularity. The pars tuberalis is more vascular than the pars intermedia and less vascular than the anterior lobe proper. Its cellular arrangement is that of cell masses with occasional small, relatively thick-walled acini. The cells are basophilic with rather scanty cytoplasm.

The histological features of the three parts of the reptilian hypophysis are thus summarized by Baumgartner ('16):

The pars intermedia has a laminar arrangement of columnar clear-staining cells. The part derived from the lateral lobes are arranged

in columns (or sometimes acini) of clear-staining polyhedral cells. The anterior lobe proper is formed of columns or acini, with clear-staining and darkly-staining cells which may be acidophilic or basophilic. In general, the pars intermedia and the parts derived from the lateral buds may be considered the chromophobic and the anterior lobe the chromophilic part.

Parker ('17) has called attention to the tubular or acinar structure of the pars tuberalis of the hypophysis in marsupials.

In a recent paper the writer has emphasized the differences in structure in the several parts of the rabbit's hypophysis at birth. Of considerable interest in distinguishing between the pars intermedia and the pars tuberalis are the peculiar spindle-shaped and branching cells which have been observed in the former (Retzius, Herring, Trautmann, Atwell, and others). They have been called ependymal and neurogliar elements (Stendell). It is important to note that these cells have never been described for the pars tuberalis.

The frog forms an exception to some of the features found in the Amniota. In this form the pars intermedia is fairly vascular while the pars tuberalis is non-vascular. In the specimens studied (which were obtained in October and November) the pars tuberalis does not show a tubular or acinar arrangement of its cells. The pars intermedia is clearly in contrast to the pars tuberalis on account of the numerous hyalin bodies to be found in the former, but never in the latter (R. catesbiana).

It will thus be seen that there is very good agreement among the several vertebrate classes, so far as studied, as regards the main features of structure of the three epithelial lobes.

Development. It is by careful ontogenetic studies that the distinction between the three epithelial lobes is most strikingly brought out. Thus the pars intermedia, in those forms possessing a hollow hypophysis anlage, develops from the dorsal wall of Rathke's pouch. In those forms having a solid hypophysis fundament, as the frog, the pars intermedia is derived from the dorsal or caudal tip of the solid anlage. During development, in all forms, the pars intermedia becomes intimately united with the neural lobe.

The anterior lobe proper develops from the main body of the epithelial anlage. In the frog this is the middle and anterior part of the gland with the exception of the lateral lobes. In vertebrates higher than the Amphibia the anterior lobe is formed mainly from the anterior or ventral wall of Rathke's pocket.

The pars tuberalis has a paired origin, being derived from the lateral lobes, lateral buds, or 'tuberal processes.' It had been believed that for mammals the lateral lobes appear relatively late in development (Tilney, Miller), but the writer ('18) has shown that they are present very early in rabbit embryos. It seems likely that the 'bilateral origin' of the hypophysis observed in Ambylstoma by Kingsley and Thyng is an early appearance of the anlagen for the pars tuberalis. This, at least, is my interpretation of the bilateral conditions above recorded for very young embryos of R. pipiens.

The definite lateral lobes are, during development, attached close to the epithelial stalk at the ventral or nasal end of the gland. They may fuse with one another and form a single epithelial plate closely applied to the floor of the third ventricle (most Amniota). They may maintain their attachment to the anterior lobe proper or they may become detached as cell masses (certain reptiles), or as two epithelial plaques (frog). In snakes and in some lizards it is even stated that the lateral lobes disappear entirely (Baumgartner, '16).

SUMMARY

1. The hypophysis of the Anura consists of three epithelial lobes and a neural lobe. The lobes of epithelial origin are the anterior lobe proper, the pars intermedia, and the pars tuberalis.

2. From their development and their mature structure these lobes may be considered homologous with corresponding lobes of the hypophysis in all higher vertebrates.

3. The anterior lobe proper develops from the main central portion of the solid epithelial anlage. It comes to lie caudal and ventral to the other portions of the gland.

4. The pars intermedia develops from the caudal tip of the hypophysial anlage. It forms a long transverse structure conforming to the shape and extent of the neural lobe.

5. The pars tuberalis has its origin in the lateral lobes, which appear very early. These two lateral lobes spread out under the brain floor and become detached, forming two discrete, rounded plaques lying close under the brain floor in the pia mater.

6. The pars tuberalis is a constant structure, during development, in the hypophysis of amphibia and higher vertebrates. It is characterized by its paired origin (the two lobes appear relatively early and have their attachment near that of the epithelial stalk) by its laminar nature and by its adult location in the pia mater covering the tuber cinereum of the brain floor.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, AUGUST 7

LYMPHATICS OF THE OMENTUM

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ONE FIGURE

The significance of the omentum has been since the earliest times a matter of much theory and conjecture. Various protective and other functions have been attributed to it, but these in the main have been based on theory. Only recently has there been definite experimental work to prove the accuracy of some of these assumptions. Most recently the interest has been centered on what part the omentum might play in absorption from the peritoneal cavity. Previous work on peritoneal absorption has resulted in emphasis being placed on the diaphragm as the main pathway of absorption, Adler and Meltzer, Muscatello, and others holding that drainage was by way of lymphatics, while Heidenhain, Starling and Tubby, Cohnstein, Hamberger, and Dandy and Rowntree have shown that much of the absorption takes place by way of the blood stream. However, assertions had been made by some that the omentum aided in peritoneal absorption; but not until Rubin in 1911 showed that less fluid was absorbed from the peritoneal cavities of animals whose omenta had been amputated than from the peritoneal cavities of normal controls, was there any experimental evidence to support these assertions.

Knowing the facility with which material passes from the peritoneal cavity through the diaphragmatic lymphatics, the question arose as to whether or not the omental lymphatics might play a like rôle, but only a short survey of the literature was necessary to cause the matter to resolve itself into whether or not the omentum contained lymphatics. Ranvier found them in new-born kittens, but claimed that they disappear by

retrogression or by some degenerative process by the end of the third month. Others have maintained that there are no lymphatics in the omentum, and some of the earlier workers who made very extensive studies of the lymphatic system do not mention the omentum at all as one of the lymphatic-containing organs. And just recently Shipley and Cunningham, who have made a careful study of the omentum, proving definitely that active absorption from the peritoneal cavity takes place by way of the blood stream of the omentum, were unable to demonstrate the presence of lymphatics. On the other hand, Klein, Norris, and others speak of lymphatics of the omentum, but do not give convincing proof of their existence, and it is this latter fact together with the fact that it is very difficult to adequately demonstrate them in a place where one would think they should be easily demonstrable that is responsible for the uncertainty which those feel who have had occasion to think of or look up the matter.

In my first attempts von Rechlinghausen's silver method was used. Omental spreads were made, the surface was rinsed with distilled water, a one-fourth of 1 per cent solution of silver nitrate was applied, and after one or two minutes the silver solution was rinsed off with distilled water. The specimen was then immersed in distilled water and exposed to the sunlight until it assumed a brownish tint. These silver markings were also brought out by the arc light, but less effectively. By this method I found that only the peritoneal lining of the omentum was silvered. Then I followed Klein's technique of penciling off the surface of the omental spread with a camel's-hair brush in order to remove the peritoneal covering before applying the silver nitrate. In this way beautiful rich networks of lymphatics were readily brought out in the centrum tendinum of the diaphragm just such a picture as that diagrammatically sketched by Klein for the omentum, but in no case were lymphatics found in the omentum by this method except in the new-born kitten where some of the lymph-vessels were present in the very thin meshes of the omentum. However, since the silver did not with this technique penetrate the perivascular fat and the thicker parts of the omentum around the blood-vessels,

it did not exclude the possibility of there being lymphatics in those areas. Attempts were then made to inject with color masses, but with no success. The extremely fine and delicate structure of the omentum made this method unsatisfactory. It was then decided to return to the silver method and attempt to silver the perivascular structures by forcing the solution through the blood stream under increased pressure. Immediately after killing the animal a cannula was inserted in the abdominal aorta, the aorta was then ligated below the cannula and above the coeliac axis. A nick was then made in the portal vein to insure a free outflow, and distilled water was passed through the omental vessels to wash them out. Immediately following that a one-fourth of 1 per cent silver nitrate solution was forced through the omental vessels under rather high pressure. One could readily determine the extent of injection by the immediate milky opacity assumed by the tissues which the silver invaded, and this was the guide in regard to the amount of pressure to use. The omentum was then removed, immersed in distilled water and exposed to the sunlight until the brownish tint appeared. The specimen was fixed in alcohol and was cleared by the Spalteholz method, and it was found that the solution had passed through the walls of the arteries and had invaded and silvered all structures in the perivascular areas. By this method and with local forced injections lymphatics were found in the region of the larger blood-vessels of the omentum in the rabbit, cat, dog, and in man. The lymphatic endothelium is typical and unmistakable and is easily distinguished from that of the veins and arteries by its different pattern. It must be said here, though, that in no case were the lymph-vessels numerous.

After finding the lymphatics by the above method, it seemed unreasonable that one should not be able to inject them with color suspensions, but further attempts along this line were practically without success. Whether the fact that removing the omentum from the peritoneal cavity, exposing it, and stopping its circulation which gives it a tendency to dry rapidly and contract somewhat, would cause the delicate-walled lymphatics to contract and make injection difficult is only a possible explanation.

Attempts at injection did, however, bring out one or two interesting facts. In the cat and adult pig, by making shallow injections in the ventral stomach wall along the greater curvature just anterior to the line of attachment of the omentum, one was able to get loops of lymphatics running down from 2 to 4 or 5 cm. into the omentum. From the position of these loops it would appear that the lymphatics had been dragged down from the stomach wall in the growth and development of the omentum. There was no evidence of drainage of lymphatics from the omentum proper into these loops. Also when one injected in the corresponding prepyloric region of the rabbit's stomach, the injection mass would pass from the stomach wall through a single lymph channel which coursed down through the ventral leaf of the omentum to its lower splenic attachment. This vessel had no definite relation to any of the large blood-vessels, but passed across one of the less vascular areas and could not be considered a true omental lymphatic.

Attempts at injection further brought out the peculiar pattern of the blood-vessels of the omentum. In the normal omentum capillaries are comparatively rare, but there is an unusually rich arterial and venous anastomosis.

Reverting to the main subject, at this point it was felt that I had progressed practically no further than others who had claimed positive results. Therefore, in the hope of producing absolutely definite and convincing evidence for the presence of lymphatics in the omentum, it was decided to run some absorption experiments, following the method used by Shipley and Cunningham in their peritoneal absorption experiments. Instead of decerebrating the animal, it was anaesthetized with luminol-sodium (a drug which produces an even quiet anaesthesia which can be made to last from one to six or eight hours by regulating the amounts, but from which the animal never recovers), was then placed in a box in which physiological conditions were maintained, and the omentum was carefully drawn out of the animal's body through a midline incision and was kept immersed in a carmine solution. In the several experiments various lengths of time ranging from one-half to three

hours were allowed for absorption to take place and then all the possible lymph glands to which drainage might occur (including the mediastinal glands) were removed, sectioned, and were examined for the carmine granules. Also each time a careful

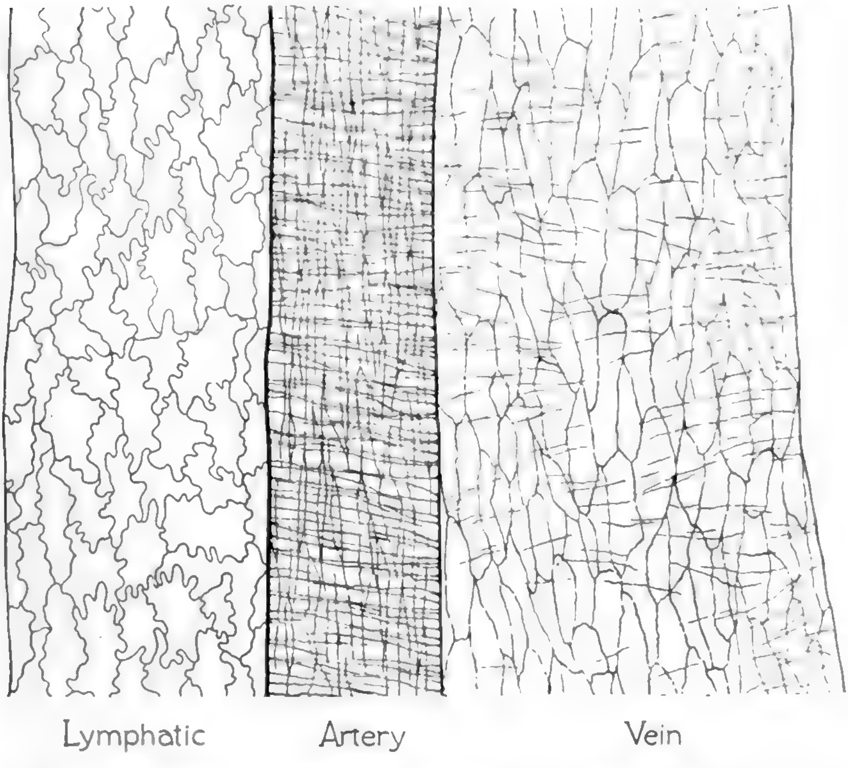

Fig. 1 Specimen of the omentum of an adult cat in which silver nitrate (0.25 per cent) was injected into the arteries to show comparison of the size and shape of the endothelial cells of an artery, vein, and lymphatic. The transverse lines on the artery and the vein indicate the smooth muscle. The entire width of the artery is not shown, as part of it was covered by the lymphatic.

search was made for an indication of the channels of absorption, but both here and in the case of the glands examined the results were negative. Then it occurred to me that possibly I had failed to find the glands or that the drainage might be direct. Additional experiments were made, lymph was withdrawn

from the cysterni chyli and smears were made, with the result that a few granules were found each time. At other times lymph was drawn from the thoracic duct proper and granules were found, but in neither case were they numerous. Other experiments were made which were similar to the above with the exception that for the absorption material a true solution was used (potassium ferrocyanide and iron ammonium citrate). Glands were removed as before and were fixed in hydrochloric acid formalin without the formation of Prussian blue, but when the lymph was drawn from the cysterni chyli and from the thoracic duct and was placed in hydrochloric acid formalin, Prussian blue was formed.

These experiments then confirm the findings with the pressure injection of silver nitrate into the blood stream—they show definitely that lymphatics are present in the omentum and furnish a channel for absorptions from the peritoneal cavity. Whether the lymphatics really play a very active rôle in this latter respect I am not prepared to say, for while absorption was rather meager in the above experiments it might be considerably more pronounced when the omentum is closed up in the peritoneal cavity where pressure conditions are necessarily different, or the meager absorption might have been due to the small number of lymphatics present. The experiments also show that drainage from the omental lymphatics is into the cysterni chyli and on through the thoracic duct and not by way of the posterior mediastinal glands as hypothecated by Crouse in his extensive paper on the great omentum.

I wish to take this opportunity to thank Dr. F. R. Sabin, Professor of Histology in the Johns Hopkins Medical School, also Dr. R. S. Cunningham, Instructor in Anatomy in the same institution, for their helpful suggestions and criticisms and also for placing equipment at my disposal.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, AUGUST 7

AN UNUSUAL RIGHT LUNG

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In the course of the regular dissection an unusual right lung was found recently. In brief, its interesting features were:

1. A rudimentary middle lobe so hidden in the interlobular fissure as to make the lung appear two-lobed from a simple surface inspection, and

2. An unusual distribution of the second lateral bronchus.

The inferior interlobular fissure did not reach the diaphragmatic surface, but turned around the ventral margin on to the mediastinal surface and thence dorsalward and slightly upward to the hilus. The superior interlobular fissure separating the middle and superior lobes was not evident from the mediastinal surface; that is, there was no evidence of a middle lobe from surface inspection of the mediastinal surface of the lung. On widely divaricating the inferior interlobular fissure, the middle lobe could be seen near the mediastinal surface, lying in a position ventral to a frontal plane passing through the hilus. It was shaped as a biconvex disc about 1 cm. thick, elongated in a dorsoventral direction, with its medial edge very close to and parallel with the mediastinal surface of the lung, and its lateral edge roughly parallel to the medial edge and about 4 cm. from the mediastinal surface of the lung. The dorsoventral length was about 5 cm. It was attached dorsomedially to the hilus, the remainder being covered by pleura except the ventral extremity, which was fused with the superior lobe. Thus it was incompletely separated from the upper lobe by the superior interlobular fissure.

The relations of the structures at the hilus were those of a normal right lung. When traced deeply into the lung substance the relations of the eparterial bronchus and vessels of the superior

lobe were found to be normal. The second lateral bronchus, the normal bronchial supply of the middle lobe, was observed, however, to send a large branch to the upper lobe, taking a path through the fusion between the middle and superior lobes, while it supplied only a small branch to the middle lobe. The main-stem branches proceeded into the inferior lobe in the usual manner.

The tendency of the middle lobe to fuse with the superior lobe is well known. The diversion of the distribution of the larger part of the second lateral bronchus to the upper lobe in this case seems to suggest there is some parallelism between the extent of fusion of these two lobes and the distribution of the second lateral bronchus to the superior lobe.

This lung merits recording since: 1) it shows an extreme variation in the relations of the interlobular fissures of the right lung, and, 2) it presents a well-marked case in which an upper lobe of the right lung receives the major part of the bronchial distribution of the second lateral bronchus, and the middle lobe receives the minor part.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, SEPTEMBER 16

FURTHER STUDIES ON THE REACTIONS OF BLOOD- AND TISSUE-CELLS TO ACID COLLOIDAL DYES[1]

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SIX FIGURES (TWO PLATES)

It has been claimed that lymphocytes and leucocytes of the blood will not take up colloidal dyes in animals stained intra vitam. It is also generally believed that the reaction to colloidal dyes is specific and that it serves to distinguish between lymphoid wandering cells and fixed cells of the connective tissue and those which have migrated into the tissues from the blood.

At the New York meeting (1916) of the American Association of Anatomists the writer showed that blood-cells, both lymphocytes and polymorphonuclear leucocytes, will take up the dye and store it in the form of granules if the cells are isolated from the blood stream. It was shown that if the dye is injected into a blood-vessel which has been isolated from the general circulation by a double ligature and the vessel is left in situ for twenty-four hours, it will be found that the polymorphonuclears have stored the dye in large quantities. The lymphocytes of the vessel have also taken up the dye. Another experiment described at that time consisted of the injection of the dye into the intermuscular connective tissue. Sections of the muscle, which were removed on the following day, contained many polymorphonuclears with dye granules. These results were described in detail in a paper published in Vol. 12 of The Anatomical Record.

In that paper observations were described which gave further support to the view of Evans and Schulemann, that the cellular

[1] Aided by a grant from the Research Funds of the University of Minnesota.

reactions find their explanation in the colloidal nature of the dyes. According to these authors, the dye particles are taken into the cell by phagocytosis and concentrated in cytoplasmic vacuoles in smaller or larger masses which in the end-stages appear as more or less definite granules. The writer reviewed some of the literature on phagocytosis and showed that the reactions of the cells which are primarily concerned in intra vitam 'staining' with the colloidal dyes are exactly what should be expected if the process is one of ingestion and storage rather than of true staining of preformed cytoplasmic structures. Phagocytosis on the part of leucocytes is of rare occurrence within the general blood stream, but in the tissues and in blood sinuses, where the velocity of the current is greatly reduced, the same leucocytes may be very active phagocytes. It was shown that in their behavior towards the colloidal dyes the leucocytes follow this same general rule. The conclusion was reached that the reaction is no more specific than is general phagocytosis, and that it does not serve as a means of distinguishing between lymphoid wandering cells of histogenous origin and those which have wandered into the tissues from the blood.

These studies have been continued during the past year with results which add further support to the conclusions reached in the first paper.

In this later work it has been found that the study of the very early reactions following single intravenous or subcutaneous injections is of very great importance. This phase of the work seems to have been neglected by nearly everyone who has experimented with these dyes. The bulk of the present paper is devoted to an account of these early reactions.

It has been found that the polymorphonuclear leucocytes are much more active phagocytes for the dyes than was evident from the first series of experiments. In order to demonstrate polymorphonuclears in the blood which contained dye it was necessary to inject the dye into a segment of a vessel which had been isolated from the general circulation by means of two ligatures. Since then it has been found that it is not necessary to ligature the vessel before the injection is given if the blood is removed

from the vessel within from one-half to two hours after the injection, or if a segment of a large vessel is tied off and fixed within this time limit.

Rabbits were injected with from 20 to 50 cc. of 5 per cent Trypanblau in distilled water or normal salt solution through the marginal ear vein and fresh blood smears examined, or a segment of the inferior vena cava removed within the favorable time limit. Spleen, lung, and mesenteric lymph node were also removed at the same time and fixed in Helly's Zenker-formol mixture. Sixteen rabbits were treated in this way and, with two exceptions, polymorphonuclears containing dye granules were found either in the blood or organs, and usually in all of these locations.

The brief period of time between the beginning of the injection and the removal of the blood and organs probably accounts for the fact that the dye granules in the polymorphonuclear leucocytes are usually smaller and frequently paler than is the case when the dye is injected between two ligatures and the vessel left in situ for from nineteen to twenty-four hours. In the sections, and especially in the fresh preparations, one frequently gets the impression that the dye has stained or has been concentrated on the surface of the special granules of the leucocytes. In the later stages, in the case of the doubly ligatured vessels, or when the injection has been continued after the death of the animal, the dye granules are much larger and more irregular in shape than the special granules. The appearance of the granules in the early stages, together with the fact that it has never been possible to stain the special granules in leucocytes containing dye granules, seems to indicate that the dye granule is built up about the leucocyte granule, the latter serving as a nucleus for the larger dye granule.

There is some variation in the results of these experiments which is difficult to explain. In some cases no dye granules could be seen in any of the leucocytes of the blood or sectioned blood-vessel, but examination of sections of the organs would show that the polymorphonuclears of the capillaries or interstitial tissue of the lung, spleen pulp, or sinusoids of the liver contained beautiful

dye granules. In some other cases the blue granules were found in the blood, but not in the organs. The usual condition, however, was to find blue granules in the leucocytes of all of these locations.

All of those cells which Evans has grouped under the term 'macrophages' (histiocytes of Aschoff-Kiyono) do not take up the dye until later, when most of it has disappeared from the polymorphonuclears, which shows that the latter are more actively phagocytic for the dye than are the former.

When the blood or organs are examined later than two and one-half hours after the injection it is difficult to find polymorphonuclears containing dye granules. An explanation for this is not difficult to find. Immediately after the injection the leucocytes are floating in a solution of the dye, the concentration of which is relatively high. Consequently the cytoplasm of the leucocytes is in contact with innumerable particles of the dye, and owing to its phagocytic properties is able to take in the dye particles. The dye being very diffusible, its concentration in the plasma is gradually being lowered by diffusion into the tissues.

Evans has shown that the colloidal dyes readily pass from a region of high concentration to one of lower concentration. This probably explains the diffusion from the plasma to the tissue fluids, and also from the polymorphonuclears to the plasma, as soon as the concentration of the latter has been materially reduced. In the circulating blood the polymorphonuclears are in contact with the dye during a relatively short period of time, and hence the dye which they have taken up is not so firmly anchored as it is in the case of the doubly ligatured vessel from which diffusion takes place very slowly, resulting in the storage of more dye and the formation of more resistant dye granules. In the latter experiment the dye granules of the leucocytes are very large and brilliantly colored after twenty-four hours, while the leucocytes of the circulating blood contain no dye granules at this time.

In case the death of the animal occurs during the injection, the conditions of the doubly ligatured vessel are duplicated. The circulation is stopped and diffusion from the vessels is very much reduced. The leucocytes, of course, continue to live for some time, and being in intimate contact with a high concentration of

the dye in the quiescent state they are able to phagocytose more of it than would be possible in the circulating blood stream.

The complete protocols of the two animals which died during the injection of Pyrrholblau may be of interest. Accidents of this sort rarely occur when Trypanblau is used, but with Pyrrholblau one must be prepared to lose some of the animals. In this case the accidents served a good purpose, for they gave further insight into the factors governing the ingestion of the dyes.

Rabbit 27. 50 cc. of a fresh 1 per cent Pyrrholblau solution in normal salt solution was injected into the ear vein and into both femoral veins. Total time for the injections: one hour and twenty minutes. Animal died one hour and ten minutes after the beginning of the injection. 20 cc. injected after death. Organs removed fifteen minutes postmortem.

Liver: Many polymorphonulears, including those in the larger vessels, contain dye granules. No dye in the stellate cells.

Vena cava (section): Large and brilliant dye granules in nearly all of the polymorphonuclears.

Spleen: Very hyperemic. Some free dye, but none in cells.

Mesenteric lymph node: Negative.

Rabbit 29. 14 cc. of 1 per cent Pyrrholblau in normal salt solution. Animal died fifteen minutes after the beginning of the injection. Injection stopped at time of death. Organs removed two and one-half hours later.

Vena cava: The dye has settled on one side of the vessel. All of the polymorphonuclears which are surrounded by the dye contain large and brilliant granules.

Lung: Large numbers of polymorphonuclears in the capillaries and interstitial tissue. All of them are filled with very conspicuous dye granules.

Liver: Negative.

In the material from both of these animals more of the polymorphonuclears contain dye and the granules are larger and of greater density than is usually the case when the animals are allowed to live until the organs are removed. In other words, the conditions are more like those obtained by isolation of a segment of a vessel by double ligature. Reduced diffusibility

from the vessels and the quiescent state of the dye-plasma solution are probably the determining factors in both cases.

Although the later series of experiments have shown that absolute isolation from the blood stream is not necessary for ingestion of the dye on the part of the blood-cells, it still remains evident that a slowing down of the blood current favors phagocytosis. This probably explains the few cases of early stages in which dye granules were found in the polymorphonuclears of the lung and spleen, but not in those of the vena cava. However, it does not explain the condition in one animal in which polymorphonuclears containing dye were found in the vena cava and not in the organs. The number of polymorphonuclear leucocytes appearing in the sections of lung and spleen is extremely variable in the different animals. In this case the organs contained very few of these cells, and it is possible that if a greater number of sections had been studied some polymorphs with dye granules could have been found.

In the first paper of this series (Anat. Rec., vol. 12) the writer reviewed some of the literature dealing with the subject of phagocytosis of living bacteria following intravenous injection. There is general agreement among the investigators of this subject that the organisms disappear from the circulation within a few minutes after the injection. The reticular cells of the spleen pulp and bone-marrow, and especially the stellate cells lining the sinusoids of the liver, are the most active agents in the removal of the organisms from the blood. Polymorphonuclear leucocytes may take up the organisms, but only in those locations in which the blood current is slowed down (sinusoids of liver and suprarenal body, etc.), or after the organisms have invaded the tissues. They are never able to phagocytose the bacteria in the general circulation in the larger vessels.

In order to gain some personal experience with living organisms the writer injected an emulsion of staphylococci into the ear vein of two rabbits. The results agree with what has already been reported in the literature.

The first rabbit was killed fifteen minutes after the injection. Blood smears and sections from the vena cava were negative.

The spleen contained cocci in its reticular cells, but the mesenteric lymph node was negative. In the lung cocci were found in macrophages and in polymorphonuclears. The organisms were more abundant in the liver than in any of the other organs. They were found in stellate cells and polymorphonuclears of the sinusoids in the neighborhood of the larger bile ducts, i.e., in the peripheral portion of the hepatic lobules.

In the second rabbit the inferior vena cava and portal vein were tied off and the organs removed about thirty minutes after the injection. Results were similar to those of the first rabbit. Blood smears and sections of the vessels were negative. In the liver the stellate cells and polymorphonuclears which contained cocci showed the same grouping as in the first animal, i.e., they were in the peripheral portion of the hepatic lobules in the immediate neighborhood of the interlobular vessels and bile ducts. There were not many cocci in the spleen, but a few reticular cells and polymorphonuclear leucocytes contained them. In the lung the polymorphonuclears were very numerous, and many of them contained cocci.

The results of the experiments of these two rabbits are quite similar to those obtained with the intravenous injection of colloidal dyes. In the circulating blood of the larger vessels, however, an important difference is noted which requires explanation.

Study of the blood smears showed that the cocci are removed from the circulation within a very few minutes after the injection. This fact, together with the mechanical factor of the rapid circulation of the blood through the larger vessels, probably accounts for the absence of phagocytosis in the general blood stream.

Colloidal dyes are not eliminated so rapidly, and consequently the leucocytes are floating in the dye for a relatively long period of time (one-half to two and one-half hours), and they are coming in contact with innumerable particles of the dye during this period, and hence are able to phagocytose it. In regions of slow circulation and in the tissues the leucocytes are able to phagocytose both the dye and the cocci. When escape of dye and cocci

is prevented by the double ligaturing of a vessel, phagocytosis on the part of the leucocytes is still more active. For the cocci this was checked by the injection of an emulsion of staphylococci into the doubly ligatured jugular vein of a living rabbit. The vein was left in situ for 15 minutes and then removed and fixed in Helly's fluid. In sections of the vein the polymorphonuclear leucocytes were seen to contain many cocci.

Before leaving the subect of experiments with intravenous injections of colloidal dyes it may be well to add a few selected protocols of animals kept alive until the organs and blood were removed.

Rabbit 24. 20 cc. of 1 per cent Trypanblau. Animal killed one-half hour later. Inferior vena cava tied off and fixed in Helly's fluid. Lymph node and spleen fixed at the same time. Some polymorphonuclears of the vena cava contain dye granules, but none were found in the spleen or lymph node.

Rabbit 35. 38 cc. of 5 per cent Trypanblau in normal salt. Organs removed thirty-five minutes after beginning of the injection.

Vena cava: Dye granules in the polymorphonuclears. One large mononuclear with dye granules.

Lung: Very few polymorphonuclears. They all contain pale blue granules.

Spleen: Negative.

Liver: A few stellate cells with dye granules.

Rabbit 36. 58 cc. of 5 per cent Trypanblau in normal salt. Organs removed one-half hour after the beginning of injection.

Vena cava: Dye granules in polymorphonuclears. Many of the granules are very large.

Lung: Rather pale dye granules in some of the polymorphonuclears.

Spleen and liver: Negative.

Liver: Very little dye in the organ. Some stellate cells have traces of it.

Rabbit 25. 40 cc. of 1 per cent Trypanblau. 20 cc. injected into each ear vein. Animal killed one hour after beginning of injection, thirty-five minutes after completion. Ligatured portal vein and spleen fixed in Helly. Dye granules in the polymorphonuclears of the vein, and the spleen contains a few polymorphs with dye granules.

Rabbit 26. 22 cc. of 1 per cent Pyrrholblau in normal salt solution. Animal killed two hours after the completion of the injection.

Spleen: Dye granules in polymorphonuclears, but very little dye in the reticular cells.

Liver: Dye in many of the stellate cells and in a few polymorphonuclears, but it is usually not in the form of definite granules.

Mesenteric lymph node: Very little dye in the node. A few polymorphs with dye granules. No dye in the reticular cells. A few histiocytes containing dye in the form of rounded masses of granules.

Lung: Great numbers of polymorphs with dye granules. A few small medium-sized lymphocytes also contain dye granules, but there are no granules in the larger macrophages.

Vena cava: Some of the polymorphs have pale blue cytoplasm, but in none of them is the dye concentrated in the form of granules.

Rabbit 32. 50 cc. of 5 per cent Trypanblau in normal salt. Material removed two hours after the beginning of the injection.

Spleen: Dye granules in many cells of the reticulum and in many large macrophages.

Vena cava: Very few polymorphs in the sections, but most of them contain dye granules.

Lung: Not many polymorphs, but they contain distinct dye granules.

Liver: Dye in stellate cells. No polymorphonuclears.

Mesenteric node: No dye granules in reticulum, but some reticular nuclei are blue, and some lymphocytes have blue nuclei or diffusely stained cytoplasm.

Rabbit 33. 40 cc. 5 per cent Trypanblau in normal salt. Killed two hours and twenty minutes after the beginning of injection.

Spleen: Dye in the cells of the reticulum, but the total quantity of dye in the organ is small.

Vena cava: Dye granules in the polymorphonuclears.

Lung: Some free dye, but none of it in the polymorphonuclears or other cells.

Liver: Many dye granules in the stellate cells.

The above protocols are sufficient in number to give an adequate idea of the results obtained. It will be noted that there is some variation in the results even where the time elapsing between the beginning of the injection and the removal of the organs and blood was the same. Difference in the amount of dye injected does not seem to account for these variations.

When the experiments were first begun the dyes were given in aqueous solutions, but the hemolysis resulting seemed to be a disturbing factor. For this reason the dyes were suspended in normal salt solution for the later experiments. This does not affect the storge of dye in the cells, but the amount of pigment in the organs seemed to be less when the salt solution was used.

At first it was found impossible to demonstrate dye granules in the polymorphonuclears of blood smears, even though sections of the same veins from which the blood was obtained showed clearly that the polymorphonuclears contained dye Later it was found that the technique of staining dissolved out the dye. When fresh preparations were examined without any stain the dye granules could be seen very clearly, especially when the preparation was examined by the light of a small Spencer lamp placed close to the mirror of the microscope. The heat from the lamp was sufficient to permit active ameboid motion. In these active cells the blue granules could be followed as they circulated through the cytoplasm and into the pseudopodia.

These preparations can be preserved if they are fixed in heat or in the fumes of full-strength formalin and their nuclei can be stained with methyl green, which does not affect the dye granules. Mounting the smears dry, with just sufficient damar around the

edge of the cover to support it, improves the preservation of the granules.

As has already been stated, the granules in the polymorphonuclears of fresh smears of the blood from these early stages are frequently very small. In such cases one gets the impression that they are the 'special' granules of the leucocytes which have taken up the dye. However, the very coarse and dense granules which are often seen, especially in the sections of the vessels from these stages, are identical with the typical dye granules of the macrophages of the tissues. Granules of this type are abundant in the polymorphonuclears of smears from blood or peritoneal fluid which have held the dye in suspension for a longer period of time. Such an experiment with peritoneal fluid will be described later.

In the earlier experiments, described in the first paper of this series, attempts to get dye granules in the polmorphonuclears of the subcutaneous tissue, following subcutaneous injections of the dyes, were unsuccessful. However, good results were obtained with intermuscular injections, which proved that polymorphonuclears that had migrated into the tissues could take up the dyes under certain conditions.

From the earlier experiments it was evident that a polymorphonuclear reaction always follows the subcutaneous injections. But, although there had been no difficulty in getting the polymorphonuclears to migrate into the tissues, it had been impossible to get them to take up the dye, even though the tissue was saturated with it. The second set of experiments was undertaken mainly for the purpose of clearing up this difficulty. That some progress was made will be shown in the following.

At first the later stages were studied, usually after repeated subcutaneous injections of 1 per cent Pyrrholblau, 5 per cent Trypanblau, or of lithium carmine, filtered and usually diluted one-half with distilled water. The other two dyes were also always filtered before being injected intravenously or subcutaneously. This is important in view of the fact that it has been claimed that any considerable difference in the size of the particles of the suspension will vitiate the results.

Polymorphonuclear leucocytes were seen in many of these preparations, but always without dye granules. The macrophages or histiocytes (Evans, Kiyono) and fibroblasts seemed to have taken up all of the dye which was not bound to the elastic and white fibers.

The early stages, taken within a few hours after a single injection, gave no better results. The tissue frequently contained polymorphonuclears, but they were without dye granules. One significant fact however, was noted in these earliest stages, and that was that the elastic fibers were stained very brilliantly (Evans) and that the white fibers had also taken up a great deal of the dye. Various cells of the tissue had also stained diffusely, but no macrophages had deposited the dye in granular form. It was these observations which determined the continuation of the experiments.

The next step was to get a series of continuous stages, beginning with one hour after injection and taken at definite intervals up to the time that the polymorphonuclears disappear from the tissues and the dye becomes deposited in granular form in the macrophages and fibroblasts. It was thought deirable to get as many of these stages as possible from a single animal and following a single subcutaneous injection, or several injections given at the same time.

The technique for this is very simple. A few cubic centimeters of the dye are injected into the subcutaneous tissue by means of a 'Record' syringe. When it is desired to examine the tissue, a slit is made in the skin in the region of injection. With fine forceps a small bit of the softer part of the tissue is lifted up and cut out. This is then spread out as thinly as possible on a coverglass by means of smooth, fine needles. Mopping up the excess fluid with filter-paper will greatly aid in this operation, as it will cause the tissue to adhere more firmly to the cover. Before the preparation has completely dried the cover is floated on Helly's Zenker-formol mixture and fixed for from one-half to one hour. The cover and adhering tissue is then washed in running water for two hours. The tissue is gradually dehydrated and iodine added when 70 or 80 per cent alcohol has been reached. Ziehl's

carbol-fuchsin is a good stain for these preparations, as it does not change the color of the dye granules.

The following stages have been studied: 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 24, 30, 36, 48, 116, etc., hours up to several weeks. Not all of these stages were obtained in any one rat. The two- to six-hour series was run in each of three rats, and the eight- to twenty-four-hour series in each of two rats. Different rats were used for the later stages, and some of the earlier and more important stages were obtained at different times from different rats.

Study of the material from these series soon gave an explanation for the absence of dye granules in the polymorphonuclears of the previous experiments. It was found that the subcutaneous injection of the dye was always followed by the migration of numerous polymorphonuclear leucocytes from the vessels, but that the time at which this took place was subject to considerable variation in the different animals. In some cases it occurred as early as five or six hours after the injection, but in others these cells were not found until the twenty- or twenty-four-hour stage had been reached.

If the reaction occurred during the earlier periods the polymorphonuclears contained no dye granules, and this is also true of those cells which were found in the tissue later than the twenty-four hour period. When the polymorphonuclears were numerous at the 12-, 16-, 20-, and 24-hour periods they generally contained dye-granules, and it seemed to make little difference whether Pyrrholblau, Trypanblau, or lithium carmine had been used. In one of the most complete series numerous polymorphs containing dye granules were found at the 12-, 16-, 18-, and 24-hour periods. In this series it was quite evident that the number of cells containing granules was being rapidly reduced in the later stages. In the twenty-four hour stage there were still many polymorphs in the tissue, but very few of them contained granules. After this time these cells were seen to degenerate rapidly, and in a few more hours they had completely disappeared from the tissue. By this time most of the dye had been concentrated in the histiocytes (fig. 3).

The phenomenon seems to be explained by facts which have already been partly described by Evans and others. It is evident from the above that the time at which the polymorphonuclears appear is the most important factor. This is related to the well-known fact that in the early stages the dye diffuses through the tissue, staining some of its elements from which it is later released. The storage in the histiocytes and fibroblasts does not take place until later, although the beginning of this process was seen in several instances as early as five and six hours after the injection.

Figure 2 represents a portion of a field from a three-hour stage for which no counterstain was used and figure 1 represents a fourteen-hour stage, stained with carbol-fuchsin. It is quite evident that all of the elements of the tissue have stained intensely with the Trypanblau which was used for the injection in both cases. The cells as well as the fibers have taken up the dye. Figure 2 shows the intense staining of the elastic fibers, and figure 1 gives an idea of the immense quantities of the dye which may be absorbed by the lymphoid wandering cells of the tissue.

If these two figures are compared with figure 3, which is from a celloidin section of the body wall of a fifteen-day stage, it is evident that the appearance of the cells and fibers in the earlier stages is very different from what it is in the late stage of fifteen days. In the latter the dye has been stored in granular form, and there has been a tremendous multiplication of cells. The nuclei are free from dye (they are counterstained with carbol fuchsin in this preparation) and the fibers are barely visible.

Considerable shifting of the dye must have taken place between the stages represented in figures 1 and 2 and the stage shown in figure 3. Close study of the series of preparations shows this to be the case. The beginning of the storage of the dye in granular form is frequently seen as early as five or six hours, but at this stage the number of cells containing dye granules is always very small. Their number increases progressively in the later stages, while the cells and fibers which were stained with it in the earlier stages gradually grow paler. In other words, the dye is being liberated by those elements which had absorbed it soon after

the injection, and at the same time it is being taken up rapidly and stored in the form of granules by fibroblasts and rapidly multiplying 'macrophages.' While this process is taking place there must be a certain amount of free dye present in the tissue fluid, for if the polymorphonuclears appear at this time (twelve to twenty-four hours) they will also store it in the form of granules. If they come later the dye has already been disposed of by the fibroblasts and macrophages, and there is none available for the polymorphonuclears. If they come too early they get none of it, because it is all bound to the fibers and cellular elements of the connective tissue (figs. 1 and 2).

During the time of storage of the dye in the 'macrophages' the number of lymphocytes in the tissue increases very rapidly. One can easily trace all intermediate stages between those which contain only a few dye granules and those without dye. Aschoff, Kiyono, Evans, and others have claimed that the reactions to colloidal dyes give final proof of the existence of two distinct and independent lines of lymphoid cells. Those of lymph-adenoid (hematogenous) origin presumably never store the dye, while those of tissue origin are marked by their great dye-storing ability.

This reasoning is no more logical when applied to the lymphoid wandering cells of the connective tissue than it is for the fibroblasts or polymorphonuclears. For the fibroblast Kiyono admits this very freely. He cites Ribbert as finding that the number of dye granules in the fibroblasts depended on the amount of injected dye solution. After from six to eight injections most of the fibrolasts contained granules, but there still remained a few non-granular fibroblasts. With direct subcutaneous injections they all contained granules, but the number of granules in the different cells was still extremely variable.

In commenting on this, Kiyono concludes that the occurrence of the red granules (lithium carmine) depends on the concentration of the dye in the tissue fluid. Kiyono himself found that in the omentum and loose subcutaneous tissue the number of granules in the fibroblasts is extremely variable. In areas of inflammation he found that the fibroblasts which had become rounded

off contain more numerous and coarser granules, which he believes is related to the stronger nutritive stream through these cells. His conclusion is, that the intensity of the carmine granulation differs according to the functional condition of the fibroblasts.

To the writer these two conclusions of Kiyono seem quite logical and well supported by facts. However, when Kiyono will not admit the application of the same reasoning to the lymphocytes he is surely on very unsafe ground.

In the first paper of this series the writer showed that lymphocytes of a doubly ligatured vessel may contain dye granules, and in this later study of the subcutaneous tissue the same fact is very evident. Tschaschin and Maximow have also come to the same conclusion, the former from a study of the subcutaneous tissue, and Maximow from a study of tissue cultures from lymph nodes and spleen. Why the lymphocytes of the blood stream and lymphoid organs should not take up the dyes under ordinary conditions was explained by the writer in his first paper.

Not all of the polymorphonuclears which arrive in the tissue during the favorable time contain dye granules. If we apply the reasoning of Aschoff-Kiyono to them we must conclude that there are two kinds of polymorphonuclear special leucocytes, a conclusion which even these gentlemen would hardly venture.

The blood-vessels of the subcutaneous tissue are very active in taking up the injected dyes, as is seen from figure 4, which is taken from the subcutaneous tissue of a rat injected with lithium carmine. This will be discussed in more detail later, but for the present it is of interest to note that the polymorphonuclears within these vessels are frequently gorged with the dye. This is shown very clearly in figure 4, where the polymorphonuclears are filled with large masses of the carmine. Exactly the same results were obtained with Trypanblau.

At the Minneapolis meeting (1917) of the American Association of Anatomists, Addison and Trorington gave a paper on the effects of intraperitoneal injection of Trypanblau. They found numerous polymorphonuclears in the peritoneal fluid, but none of them contained dye, while many of the mononuclears did contain it.

In the discussion of this paper the writer suggested that the polymorphonuclears probably would have taken up the dye if it had been present in sufficient quantities at the time of their arrival. The dye disappears very quickly from the peritoneal cavity. Much of it is absorbed directly by the blood-vessels of the omentum (Shipley and Cunningham) and the lymphatics of the central tendon of the diaphragm, and some is stored by the cells of the tâches laiteuses, clasmatocytes, etc., of the omentum and by the mononuclear cells which are normally present in the peritoneal fluid. Unless the polymorphonuclears arrive very soon after the injection they will find very little free dye.

That polymorphonuclears which migrate into the peritoneal cavity may take up Trypanblau if it is made available for them is shown by the results of the writer's experiment with rats 44 and 45. These rats were injected intraperitoneally with 5 cc. of a 5 per cent Trypanblau solution. Sixteen hours later some of the fluid was removed by means of a fine pipette and examined. It contained numerous polymorphonuclears, but they were without dye granules. Another injection of 4 cc. of the same solution was then given, and fresh preparations examined and smears made six hours later. As was to be expected, many of the polymorphonuclears now contained numerous dye granules which were identical with those of the monouclear cells. Many of the granules were surprisingly large and dense.

In order to make sure that the marked phagocytosis of the dye on the part of the polymorphonuclears was not due to the addition of salt to the solution, rat 45 was injected with Trypanblau made up in distilled water in place of the physiological salt solution used in most of the experiments. No differences could be detected between the material obtained from this rat and that obtained from rat 44 which was injected with Trypanblau made up in salt solution.

The results of this experiment seem to indicate that, in this case at least, phagocytosis does not depend so much on the size of the particles which irritate the cell membrane (Addison and Thorington) as it does on the availability of the material to be phagocytosed. It is true that the size of the object to be phagocytosed may

have something to do with the reaction in so far as particular types of cells are involved, as is indicated by Metchnikoff's classification of the leucocytes into 'macrophages' and 'microphages.' However, there are plenty of exceptions to the rule. Thus Schott describes the phatocytosis by polymorphonuclears of entire erythrocytes which were injected into the peritoneal cavity, and Rowley describes a case of anemia in which all of the leucocytes of the blood were very active in the phagocytosis of red cells. Evidently phagocytosis depends on something more than the irritation of the cell membrane by particles of varying size.

In view of what has already been reported in the literature, it is strange that some investigators still believe that the colloidal dyes cannot be phagocytosed by leucocytes. Ribbert, who was one of the first to use lithium carmine, reported that leucocytes which enter atretic ovarian follicles contain carmine granules. Pari, a student of Ribbert's, found that by ligaturing the ductus choledochus he could get polymorphonuclears in the heart blood which contained lithium carmine granules. In the same animals large lymphocytes, large mononuclears, and transitional cells also contained carmine granules. Loele, working with Isaminblau, injected one mouse which had a deposit of pus on the surfaces of the pleura, liver, and spleen due to pleuritis and peritonitis. Many of the polymorphonuclears contained blue granules. Loele's interpretation of this is interesting. He believes that the polymorphonuclears do not ordinarily stain with the colloidal dyes, because their granules are able to destroy (reduce) the dyes as rapidly as they come in contact with them. In the periotonitis mouse the leucocyte granules are stained because they are so injured by harmful agents that they are no longer able to destroy the dye.

Goldmann also observed dye granules in polymorphonuclears and large mononuclears of the blood after repeated injections of Trypanblau. However, he interprets these granules as pathologic formations, rather than as examples of true vital staining.

In experimental pneumonia Kline and Winternitz found dye granules in the involved portions of the lungs which were cut off from the general circulation by plugs of fibrin in the capillaries.

For the lymphocytes, Maximow seems to have demonstrated beyond question that they are able to take up the colloidal dyes after they have undergone further differentiation. Goldmann and Tschaschin should also be listed among those who believe that lymphocytes may phagocytose the colloidal dyes, although they both believed that the phenomenon was one of true vital staining of preformed structures or of structures formed under the influence of the dyes.

While hunting through the tissues of different stages for polymorphonuclears containing dye granules, some interesting observations were made on the early reactions of the tissue to the dyes. Some of these observations do not agree with what has been previously reported in the literature. This is because most of the previous work has been confined to a study of the later stages, especially after the animals had been given repeated injections of the dyes.

It has been generally assumed that diffuse staining of the cells, especially of their nuclei, is always an indication of a pathologic condition of the cells or of their death. Pari and others have shown that injured cells stain diffusely with lithium carmine, but according to Pari dead cells do not stain at all with the carmine. These observations, together with those of Goldman, Kiyono, Evans, etc., all apply to the later stages.

If this rule holds good for the earlier stages, then we are forced to conclude that Trypanblau is quite toxic for all of the cells of the connective tissue, for in the earlier stages they all stain more or less intensely with the dye. Especially the nuclei show a great affinity for the dye soon after it is injected. Fibroblast nuclei are frequently stained when their cytoplam contains none of the dye (figs. 1 and 2, *Fbl.*). The free wandering cells of the tissue and the clasmatocytes usually absorb much more of the dye, as is shown especially well in the fourteen-hour stage from which figure 1 was drawn.

The upper, large cell of figure 2 shows the beginning of a process which is more marked in the later stages, especially in the fourteen-hour stage of figure 1. The nuclei of the free cells are very dark from the large quantities of dye which they have ab-

sorbed. The peripheral portion of the cytoplasm is also very dark, but that part of it which immediately surrounds the nucleus is comparatively free from dye. The picture resembles the so-called Hünefeld-Hensen pictures obtained when 12 per cent cane sugar is added to Amphibian blood. Meves explains the resulting changes of the erythrocytes as follows. The sugar solution produces a firm precipitation membrane at the surface of the erythoeytes, while the hemoglobin in the interior of the cells remains fluid. With the death of the cell the nucleus swells, and in so doing it absorbs the more fluid hemoglobin, while the surface membrane and the marginal band prevent the collapse of the corpuscle.

The free cells of the connective tissue are evidently similarly affected by the Trypanblau, for in general appearance they are quite similar to the Amphibian erythrocytes which have been treated with a sugar solution. In 1913, the writer described somewhat similar pictures which resulted from the treatment of mast cells with aqueous solutions. The soluble metachromatic substance of the granules diffused into the nucleus just as does the hemoglobin in the case of the erythrocytes treated with sugar solution.

The cells of figure 1 seem to have passed through a similar process. The dye has probably coagulated the peripheral cytoplasm, but enough of it has diffused into the nucleus to cause the latter to swell and to absorb the surrounding more fluid portion of the cytoplasm, with the dye which it contained. This condition does not last long, for in the twenty-four-hour and later stages such cells are quite exceptional.

By no means all of the free cells are in this condition. Some of them contain very little dye, and in some it has already begun to be deposited in the form of distinct dye granules as early as five and six hours after the injection. In the late stages the number of these latter cells increases rapidly, while the number of the vacuolated cells with the dark nuclei decreases. It is difficult to say just what becomes of all of these. If they all degenerate one would expect to find far more degenerating cells in the preparation than are actually present. A few degenerating

cells are always present, even in the early stages, but their appearance is very different from that of the cells which contain so much of the dye. Their nuclei become pyknotic and stain intensely with the carbol-fuchsin which was used for a counterstain on most of the preparations, while their cytoplasm contains little or none of the colloidal dye. Such a degenerating cell is shown in figure 1, *deg.* Its nucleus is the only structure in the field which is stained with the carbol-fuchsin.

Most of the fibroblast nuclei of these early stages also contain more or less of the Trypanblau, but it is quite evident that few of these cells degenerate. Intermediate forms between the dye cells of the early stages and the dye-granule cells of the later stages could not be found, so it would seem, that most of the dye diffuses out of these cells to be deposited later in granular form by other cells. It is quite possible that some of the dye may remain in the cytoplasm of these same cells to be worked over into granules later. However, all of it eventually leaves the nucleus, and much of it is probably set free at the same time that the dye is diffusing out of the fibers, for if the polymorphonuclears reach the tissue at the time when the nucleus and cytoplasm of the cells in question have begun to grow pale they are able to get enough free dye to be able to store it in granular form.

In these early stages the endothelial cells of many of the capillaries and smaller vessels are in the condition of the large, dark, vacuolated cells of figure 1. Later they lose every trace of the dye. The statement which has often been made, that endothelial cells of the vessels do not store colloidal dyes, applies, therefore, to the later stages only.

Although it can be shown that cells like the ones illustrated in figure 1, with the nuclei filled with dye and dark peripheral cytoplasm, later give up the dye and recover, still one could hardly claim that they are normal and healthy while in this condition. It is quite likely that they have been injured by the dye, and that the staining of their nuclei is due entirely to its toxic effects. The presence of numerous cells with dense pyknotic nuclei stained deeply in carbol-fuchsin, cells which are clearly degenerating, is further evidence of the toxicity of the dye when it first

comes in contact with the tissues. It is clear that some cells are killed, and that others are able to rid themselves of the dye, especially that which has been absorbed by their nuclei, and regain their former normal appearance.

Among the cells which show the most injurious effects of the dye are the smaller wandering cells of the connective tissue and the eosinophil leucocytes, which are always abundant in the normal subcutaneous tissue of the rat. The behavior of the latter is of special interest, for in them we have a case of true staining of preformed structures which is almost invariably followed by the death of the cell.

All through the earlier stages, and in some cases even in the very late stages, granular cells were found whose nuclei were in various stages of degeneration. The most common form of these cells is illustrated in figure 6. Its nucleus is very pyknotic and stained intensely with carbol-fuchsin. The cytoplasmic granules have stained with the Trypanblau used for the injection. In many other cells of this same type the nucleus is still smaller or it has broken up into several pieces. The staining reaction of the granules varies somewhat, although in the cells with pyknotic nuclei they are always quite dark, and they never take any of the carbol-fuchsin.

Numerous cells of this type had been observed before it became possible to classify them, although it was evident from the first ones observed that they did not belong to the dye-granule cells (histiocytes, macrophages) shown in figure 3. The regularity of size and shape of the granules, and the comparative freedom of the intergranular protoplasm from dye, together with the pyknotic nucleus stained in carbol-fuchsin and the frequent perinuclear space (fig. 6), immediately placed these cells in a group by themselves. It was not until cells like the one of figure 5, and others grading from this to the type drawn in figure 6, were found that it became possible to classify this group.

The only granular leucocytes of the rat which have a ring-shaped nucleus similar to the one of figure 5 are the eosinophils. Comparison of the eosinophils of untreated normal rat tissue with the cells in question in the vitally stained animals shows that the latter are eosinophil leucocytes.

The nucleus of the eosinophils never stains with Trypanblau, but their granules are often brilliantly stained, even before the nucleus shows any degenerative changes. As the granules take more of the dye and enlarge somewhat (fig. 6) the nucleus degenerates rapidly, and finally becomes reduced to a small, dense, pyknotic sphere. This is the condition of most of the eosinophils encountered, although cells in more advanced stages of degeneration are by no means rare.

In the eosinophils we have an example of true vital staining of preformed granules with a colloidal dye. This must be clearly distinguished from phagocytosis and storage of the dye as it is illustrated in the cells of figure 3.

Since the cell of figure 6 is taken from an eight-hour stage, it is evident that the toxic action of the dye may cause very rapid degeneraton of the eosinophils. However, the cell of figure 5 was found in a preparation of a fourteen-hour stage, from which we must conclude that either the eosinophils of the injected area are not all killed at the same time or that others migrate into the region of greatest dye concentration from outlying regions of lower concentration. The large number of degenerating eosinophils in some of the later stages would seem to favor the latter view.

That true vital staining of preformed structures may occur while other cells are phagocytosing the dye and storing it in granular form is by no means a new idea. Evans and Schulemann ('15) admit this very freely when they claim that the granules of the epithelium of the plexus chorioideus, hypophysis, epithelial body, and adrenal body are vitally stained 'secretory droplets.' They compare this staining with that of the elastic fibers and cartilage matrix, for which it is not claimed that it is due to phagocytosis.

The effect of the dye on the eosinophils, the numerous degenerating cells of other types, the staining of the nuclei of the fibroblasts and lymphoid cells, together with the frequent vacuolization of the cytoplasm about the nucleus of the latter in the early stages, seem to indicate clearly that the dye has a decidedly toxic action when it is first introduced into the tissue. The eosinophils, however, seem to be the only cells which are unable to recover

from the effects of it. Later, when all of the dye has been stored in granular form in the fibroblasts and histiocytes it seems to be comparatively harmless, for degenerating cells are not so numerous, although they are never entirely absent from the preparations.

The condition of the blood-vessels of the subcutaneous tissue is of special interest in view of what has been reported for the blood-vessels of the omentum by Shipley and Cunningham. These authors proved experimentally that not only the capillaries, but also the larger veins and arteries of the omentum are very active in the absorption of solutions of foreign matter from the peritoneal cavity.

The writer's experiments with subcutaneous injections of colloidal dyes show that the vessels of this tissue possess similar absorptive powers. Most of the material which was gathered within from ten to twenty hours after the injection contains smaller and larger vessels which are filled with the dye. This conditon is of such frequent occurrence that it can hardly be due to accidental puncture of the vessels during the injection. The dye seems to pass through the endothelial cells, for in the earlier stages they are stained intensely with it. Later, as in the eighteen-hour stage illustrated in figure 4, the endothelial cells are free from dye, but there is a great deal of free dye in the lumen of the vessel, and the polymorphonuclear leucocytes of the vessel contain dye granules. The material was taken from a rat injected subcutaneously with lithium carmine. A mass of free carmine (*Car.*) is shown in the lumen of the vessel, and the polymorphonulears (*Pm.*) contain large carmine granules. The endothelial cells of the vessel are stained with the hemalum used as a counterstain, but not with the carmine.

The vessels of numerous rats injected with Trypanblau show this same condition, so it is evidently not of accidental occurrence. A mechanism for the rapid distribution of the dye to all parts of the body is, therefore, supplied by the blood-vessels. The existence of such a mechanism seems necessary in order to account for the extreme rapidity with which some of these dyes are able to reach the most remote parts of the body following single

intraperitoneal or subcutaneous injections. The part played by the blood-vessels would be surprising if the work of Shipley and Cunningham had not already demonstrated their ability to absorb foreign fluids.

SUMMARY

Polymorphonuclears are very active as phagocytes for Trypanblau and lithium carmine, but in order to demonstrate this in the general circulation the blood from the larger vessels must be examined within from one-half to two hours after the intravenous injection. Polymorphonuclears in the lung, spleen, and liver removed within this time limit may also contain dye granules before the reticular cells of these organs have gathered up any of the dye.

Polymorphonuclears in the vessels and tissues of the organs will also phagocytose living bacteria injected intravenously, but those of the general circulation will phagocytose the bacteria only when the latter are injected into a doubly ligatured vessel or after they have reached a vessel or sinus in which the velocity of the blood current is greatly reduced.

Subcutaneous injections of the dyes always cause the migration of numerous polymorphonuclears from the vessels, but the time at which this reaction takes place varies in different animals. If the leucocytes are numerous during the twelve- to twenty-four-hour period they usually contain dye-granules.

Polymorphonuclears of the peritoneal cavity will also take up Trypanblau and lithium carmine if the dyes are injected after the leucocytes have reached the peritoneal fluid.

Phagocytosis of colloidal dyes by polymorphonuclear leucocytes depends entirely on the availability of the dye rather than upon any inherent physiological difference with respect to phagocytosis between these cells and the mononuclear macrophages of the tissues.

The three colloidal dyes used in these experiments have a decidedly toxic action on the tissues when they are first introduced. All types of cells in the connective tissue show diffuse staining of cytoplasm and nucleus in the early stages. Many

cells are killed, but members of all groups of cells are able to eliminate the dye and recover, excepting the eosinophils. All of the eosinophils located in the region of the injection are killed by Trypanblau, but degenerative changes in their nuclei do not occur until after their granules have become brilliantly stained with the dye.

Blood-vessels of the subcutaneous tissue absorb large quantities of the dyes and probably carry them to other regions of the body. Endothelial cells are stained in the earlier stages, but not in the later stages when the vessels are full of dye. The polymorphonuclears within the vessels contain dye granules.

The experiments have brought additional evidence in favor of the view expressed in an earlier paper, that the intra vitam reaction to colloidal dyes is not specific, and that it does not serve to distinguish between blood-cells and tissue macrophages (histiocytes of Aschoff-Kiyono, macrophages of Evans).

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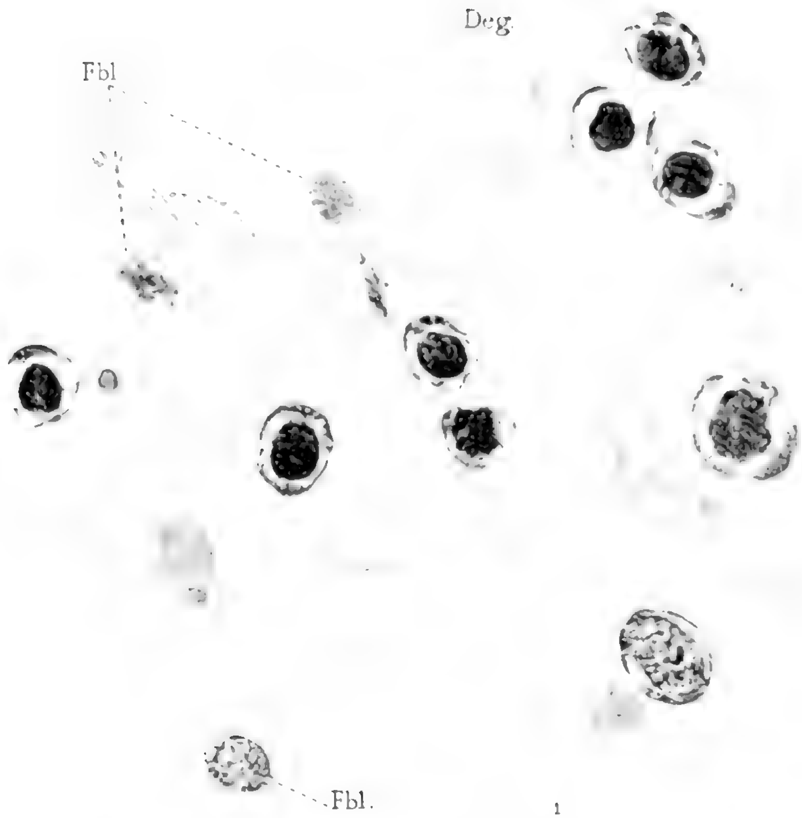
PLATE 1

EXPLANATION OF FIGURES

1 Subcutaneous tissue of rat, fourteen hours after subcutaneous injection of Trypanblau. The pyknotic nucleus of the degenerating cell, *Dcg.*, is the only structure stained with the carbol-fuchsin used as a counterstain. All of the other structures are stained with the Trypanblau. The large dark cells with the clear perinuclear space are of special interest. They are large lymphoid cells which have absorbed great quantities of the colloidal dye. *Fbl.*, fibroblast nuclei.

2 Subcutaneous tissue of rat, three hours after subcutaneous injection of Trypanblau. No counterstain. Shows staining of all of the fibers and cells of the tissue. A large lymphoid wandering cell which has absorbed a great deal of the dye is shown in the upper, left-hand portion of the figure. The nucleus of this cell and of the fibroblast (*Fbl.*) stained very dark.



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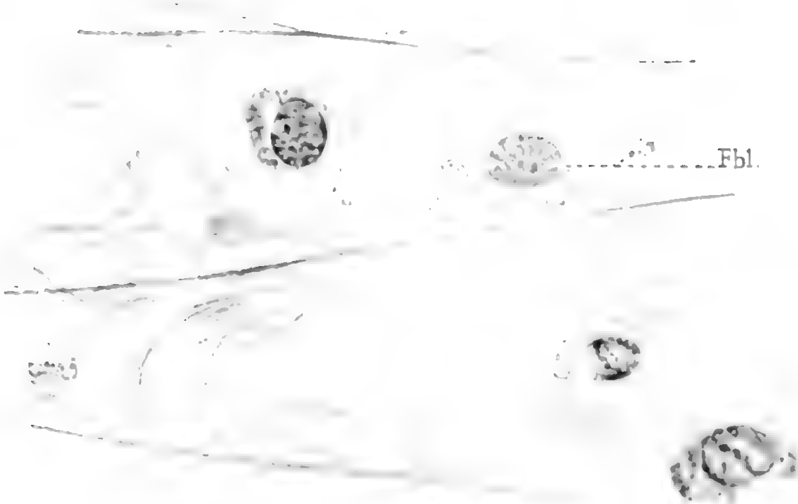

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PLATE 2

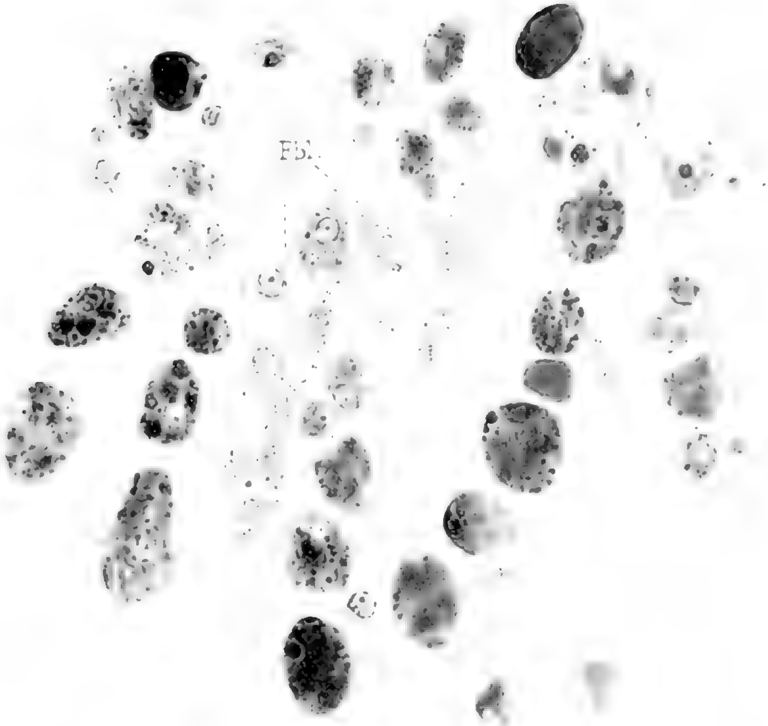
EXPLANATION OF FIGURES

3 Abdominal wall of rat. Celloidin section. Six subcutaneous injections of Trypanblau given at intervals of from two to three days. Animal killed fifteen days after first injection. Counterstained with carbol-fuchsin. Macrophages and lymphocytes of various sizes loaded with dye granules. The fibroblasts (two in the center of the field) also contain numerous fine dye granules which are very different from those of the macrophages.

4 Blood-vessel from subcutaneous tissue of rat, eighteen hours after subcutaneous injection of lithium carmine. Counterstained with hemalum. Shows absorption of the dye from the tissue. *Car.*, a mass of free carmine in the lumen of the vessel; *Pm.*, polymorphonuclears containing large carmine granules; *Ery.*, erythrocytes.

5 Eosinophil leucocyte from subcutaneous tissue of rat, eighteen hours after subcutaneous injection of Trypanblau. Counterstained with carbol-fuchsin. Granules stained blue with the Trypanblau, nucleus stained red with the fuchsin.

6 Degenerating eosinophil leucocyte with pyknotic nucleus from subcutaneous tissue of rat, eight hours after the subcutaneous injection of Trypanblau. Counterstained with carbol-fuchsin. Granules blue, nucleus red.



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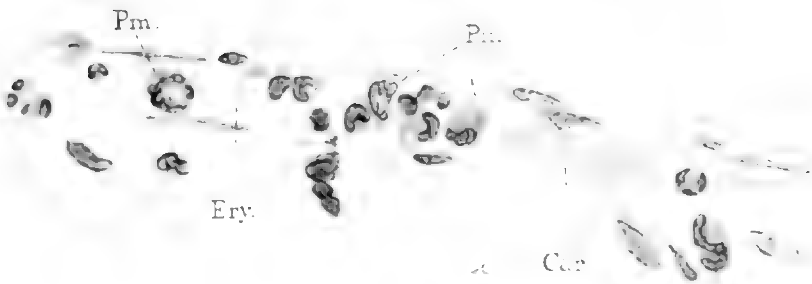

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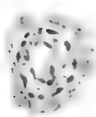

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, SEPTEMBER 16

THE EMBRYONIC STRUCTURE OF AVIAN HEART MUSCLE WITH SOME CONSIDERATIONS REGARDING ITS EARLIEST CONTRACTION

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TEN FIGURES

The long-standing differences of opinion regarding the structure of adult heart muscle have not as yet been brought any nearer to a reconciliation by the study of its simpler make-up in the embryo. In the various accounts which have appeared of the histogenesis of muscle in higher vertebrates, the myofibril has been derived in turn from granules in rows or in groups of four, from rods and filaments, from nets combined with granules, and from a honeycomb structure either combined with or free from granules. No one view has received the adherence of even the majority of the more recent writers. The observations which follow relate to the earlier stages in the development of the heart muscle and were made upon chick embryos of the first four days of incubation. They suggest a reconciliation of the divergent views regarding the early contractile structure.

Photographs were employed to measure certain elements of the sarcoplasm and also as a control to the conclusions based upon microscopical study. Several possible errors need to be considered before the trustworthiness of this method of measurement can be admitted. Spherical aberration was avoided by using only a small central area in the microscopic field. Foreshortening was readily eliminated by measuring only those planes and lines all of whose granules were in sharp focus. It is not possible, unfortunately, to do away completely with changes in the dimensions of the sarcoplasmic structures during preparation

for the slide. The usual balance was maintained, however, between the fixing agents which contract and which swell the tissue. Shrinkage due to the passage of the tissue through the paraffins was reduced to a minimum by shortening the process to four minutes. The preparations give but slight evidence of this defect.

The fixation was by Zenker's fluid or a combination of osmic acid, potassium bichromate, acetic acid, and normal salt solution. The second formula was found the more serviceable of the two. The best results were obtained with the osmic acid only when its temperature was controlled during fixation. The staining was by iron haematoxylin. Its entire manipulation was carried on in 70 per cent alcohol, as water has an unfavorable action upon the mitochondria. It was impossible to obtain entirely satisfactory fixation of the older heart because its bulk is so great as to prevent an exact control of the action of the osmic acid. The sarcoplasmic structure if well preserved is so dense that microscopic sections of 5 micra were found too opaque to give a clear view of the details. A thickness of $2\frac{1}{3}$ micra was the most satisfactory.

During the developmental period ending with the fourth day a primitive sarcoplasmic structure is present which gradually undergoes changes adapting it to its function of rhythmical pulsation. The heart muscle in ten-day chick is made up entirely of this primitive type. It can be traced back to the earliest distinguishable heart rudiment. In optical section the structure is made up of a series of granules staining with haematoxylin and connected by fine lines (figs. 1 and 2). It extends throughout the cytoplasm. The areas included by the lines are usually parallelograms approaching the form of a square. They are approximately 0.8 micron on a side. Triangles and various types of quadrilaterals also are seen.

The granules are shown well by an osmic bichromate fixation when followed by haematoxylin. The higher alcohols, oil of bergamot, and xylol partially dissolve them. Without entering into a discussion of the variations in definition of the term mitochondria, it is clear that it will be in agreement with the views of

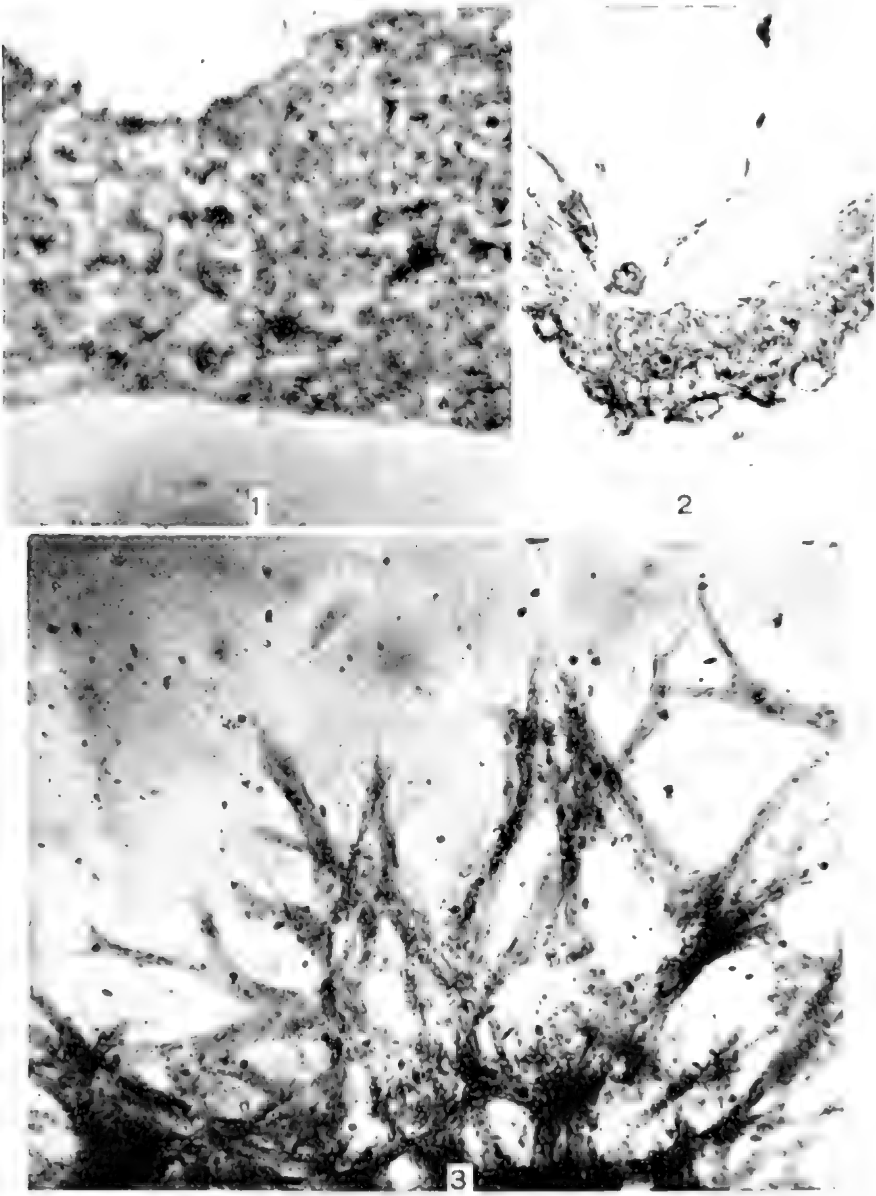


FIG. 1 Transverse section of primitive streak of chick (focused at surface of section). × 900.

FIG. 2 Transverse section of heart of a ten-somite chick. × 900.

Fig. 3 Growth in plasma culture of heart tissue from a chick of four days' incubation. × 270.

most of those occupied with the subject if the term is applied to the granules.

The network which has been described could be produced as the optical section of a mesh in three dimensions or of a system of planes completely enclosing protoplasmic bodies. The decision between these alternatives in the case of various tissues has long occupied the attention of those attempting to determine the ultimate microscopic structure of protoplasm. It is well known that the value of observations upon the finer details of fixed protoplasm as a means for determining the make-up of its living structure has been called seriously into question by the discovery that various coagulation patterns can be produced in solutions physically similar to protoplasm by the use of different conditions of fixation. It is of interest, then, that the hexahedra are found no matter whether osmic, formol, or corrosive sublimate fixing fluids are used. Also three observers have given good reasons for believing that it gradually changes during development into a structure which few would deny as an actual element of the living adult muscle, namely, the myofibril. Evidently, then, we are not dealing with an artefact and the relative likelihoods of the net and the honeycomb being the reality in the sarcoplasm has a vital interest not only in relation to the make-up of muscle, but also because of its bearing upon the ultimate structures of protoplasm.

Of the three observers who describe the network in optical section, MacCallum refers to the sarcoplasmic structure of the embryonic pig heart as a mesh. At the same time he apparently believes that it contains flat membranes, since he speaks of sarcoplasmic discs surrounded by the net. Brück also uses the word net in his discussion. Yet he definitely states that there is a honeycomb structure. Wieman believes that in the chick heart there is a net in three dimensions and applies to it the name cytoreticulum. It is not surprising that this difference of interpretation should occur when it is taken into consideration that the structure appears under the microscope in the form of lines surrounding spaces which themselves are only about 1 micron on a side. The writer is strongly of the opinion, nevertheless, that there are planes present, not filaments. The chief reason is that the lines

seem to shift instead of disappear as the focus is changed. The optical effects produced by various conditions of lighting also can be more readily explained as the action of intersecting planes than as filaments. Since one cannot see the membranes where they lie parallel to the cover-slip it must be inferred that they are too delicate to be visible except when placed nearly edgewise to the observer. A great majority of the spaces must be six-sided, since their cross-section is so uniformly a quadrilateral figure. The structure, then, can well be described as hexahedral.

CELLULAR INDEPENDENCE

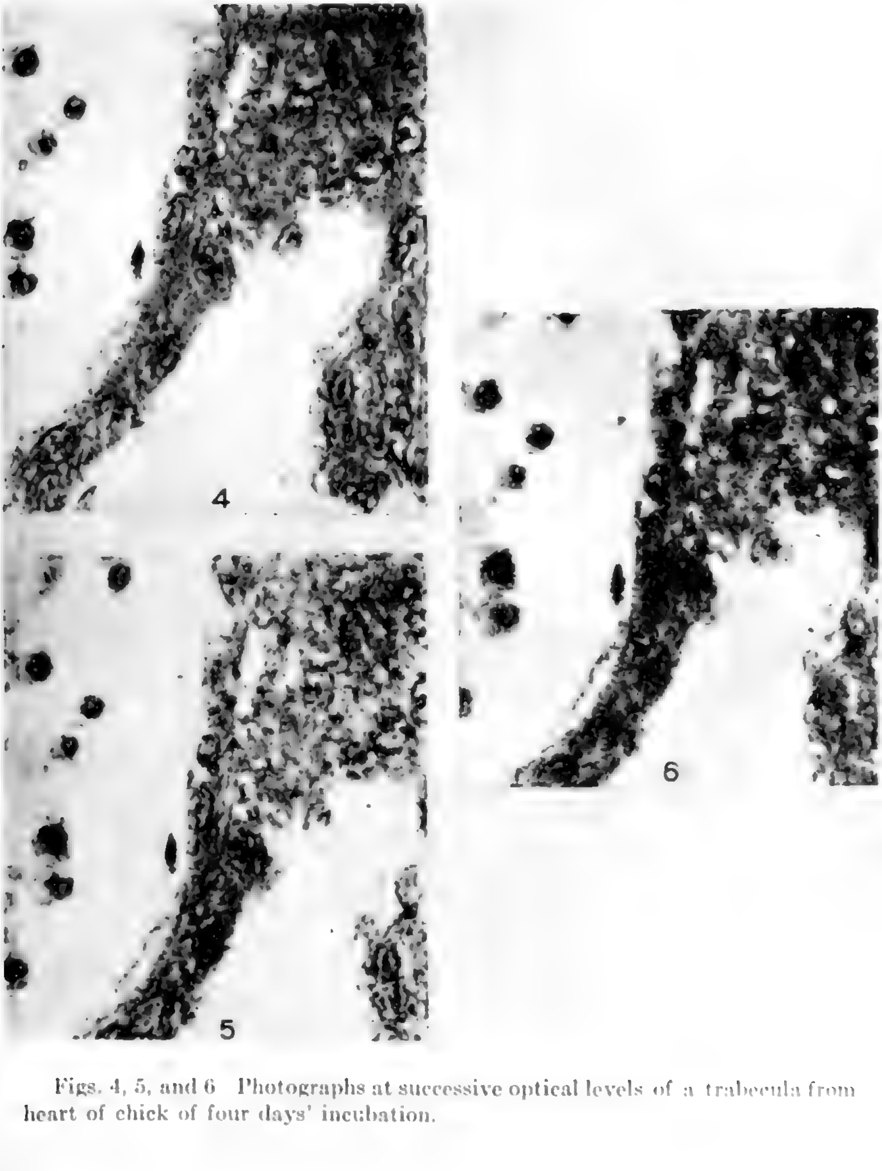
A controversy regarding the distinctness in the separation of the cell areas has been waged since the time when the cell theory was formulated. The decision in the adult heart depends on the interpretation of the intercalated discs. These do not enter into the question for the embryonic organ because they make their appearance late in development. It is generally agreed that when it is first distinguishable the heart rudiment, like the mesenchyme, is made up of cells separate except for a few fine processes. A number of observers of heart development in birds and mammals believe that they soon fuse. In this group are Chiarugi ('87), Hoyer ('01), Heidenhain ('99), Kurkiewicz ('09), and Duesberg ('10). A loss of identity is not admitted by MacCallum ('97), Wieman ('07), and Schockaert ('09).

A comparison of sections of younger and older heart ventricles indicate the possibility that there are slight distinctions between the degree of cell independence at three successive ages. At no time, however, was a cell membrane made out. In the ventricle of a ten-somite chick there is a suggestion of cellular independence consisting of an arrangement of hexahedral structure concentric to each nucleus. When a region is found, however, where the condition of the planes can be well made out all the way across from one nucleus to another, no break in their continuity is discovered or other modification to indicate a cell boundary. There is a lobation of the surface of the myocardium corresponding to each subjacent nucleus. The heart of somewhat older embryos, including

the thirteen-somite stage, contains small clefts in the interior of the myocardium partially dividing cell areas (fig. 9). Kurkiewicz ('09) has described them at length. Evidently, then, the apparently complete continuity of the sarcoplasm in the previous stage is deceptive. The four-day heart no longer contains clefts. There are portions where the planes of the hexahedral structure take a perfectly rectilinear course past several nuclei (figs. 4 to 6). If our conclusions were to be based on these localities alone there could be little doubt of the complete fusion of the cells. Other regions have a more irregular appearance.

In a previous paper ('15), which describes the migration and growth of four-day chick ventricle in plasma cultures, it was found that the sarcoplasm extended out into the clot in anastomosing multinuclear columns whose only indication of cell boundaries (fig. 3) are light constrictions of the columns. The complete cohesion of the tissue under these conditions certainly speaks for a close union between its cellular elements.

Schockaert ('09) gives the only specific statement the writer has found that there are cell membranes in the embryonic heart. She publishes a photograph of a section from a seventeen-day rabbit heart which contains markings roughly simulating cell boundaries. The finer structure of the sarcoplasm is too completely lacking to make possible an understanding of its original character. In Wieman's study of the chick heart he does not describe cell membranes, but he has obtained mononuclear fusiform sarcoplasmic bodies by maceration. These cannot be explained away as coming from regions of the atria which are tardy in their differentiation because some of them are figured with a well-developed contractile structure. Evidently, in spite of the indications of complete fusion seen in the hexahedral system and of almost complete coalescence in plasma cultures, some kind of structural demarcation between cell areas is maintained in embryonic heart. It is in harmony with this conclusion that although no cell boundaries are visible in the heart of the ten-somite chick, yet later a partial separation takes place by means of clefts.



Figs. 4, 5, and 6 Photographs at successive optical levels of a trabecula from heart of chick of four days' incubation.

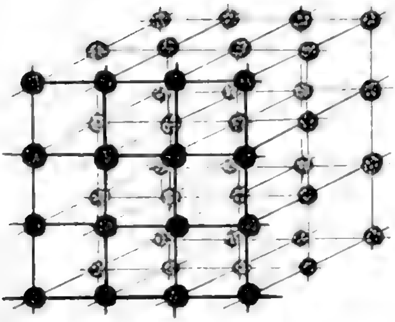
THE STRUCTURE OF THE RHYTHMICALLY-CONTRACTING HEART

An examination was made of eleven chick embryos containing from fifteen to seventeen somites to learn the time of the beginning of pulsation. The heart was beating in four which were in the seventeen-somite stage, but in none of the younger chicks.

Beginning with the heart of the sixteen-somite embryo, series of parallel bars were found in the sarcoplasm which were believed to mark areas fixed in a condition of normal function. Their formation will be best understood after the sarcoplasmic structure has been described.

The comparison of the sarcoplasm of the non-beating heart with a later stage shows surprisingly little developmental change in structure. The volume of the hexahedral spaces is still the same. The granules have increased in size. In some regions the hexahedral structure has an irregular arrangement as in the younger heart. All gradations occur between this condition and a nearly perfect alignment of the planes with intersections approaching a right angle. For the developmental period with which we are concerned alignment is best seen in longitudinal sections of the trabeculae which make up the spongy portion of the four-day ventricle. Where it is found the parallel bars usually also occur. They are associated with a uniform elongation of the hexahedral spaces in a common direction and result from a drawing together of the granules in the planes transverse to the direction of extension (fig. 8). The optical section of the spaces in extension frequently have a length of 1.2 micron and a breadth of 0.6 micron.

Contraction phases were made out less frequently than the opposite functional condition. It is possible to come upon regions, however, where the hexahedra are elongated transversely to the long axis of the trabeculae. In describing the extension and contraction only the two dimensions of the hexahedra parallel to the plane of the cover-slip have been referred to. The spaces are so small that the corresponding changes in the direction parallel to the optical axis were not satisfactorily determined. It is probable that the greater irregularity of arrangement of hexa-



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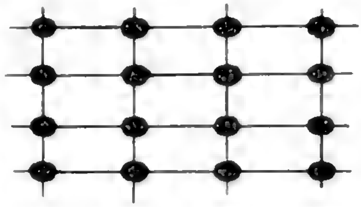

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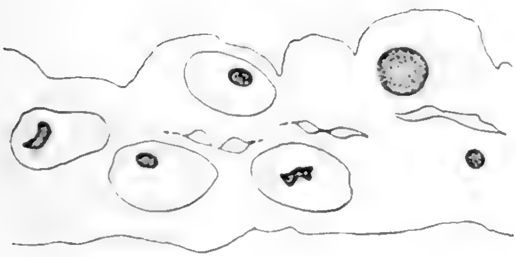

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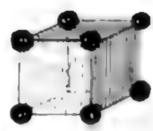

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Fig. 7 Diagram of mitochondrial granules and outlines of hexahedral spaces of sarcoplasmic structure before the beginning of rhythmic pulsation.

Fig. 8 Optical section of hexahedral structure in condition of functional elongation.

Fig. 9 Transverse section of heart wall of a thirteen-somite chick, showing clefts between cell areas.

Fig. 10 Diagram of a hexahedral space with its membranes and granules.

hedra in the compact wall than in the trabeculae is due to a less uniform direction of contraction and extension, since the wall is not only a curved plane, but has trabeculae of the spongy myocardium attaching to it in an irregular manner. The trabeculae, on the other hand, are cylinders attached only at the ends and so must contract in the direction of their long axis.

There is a certain amount of disorganization in the sarcoplasmic structure common to the wall and the trabeculae. It is probably the result of agonal contractions that have disturbed the normal pattern at the time of death. Various methods were tried in order to fix the heart without the breaking up of the regular beat into irregular local contractions, but without success. A second abnormal feature that is to be seen in some regions of the heart is taken to be the effect of unsatisfactory killing and fixing. It consists of a massing together of parallel planes into long bands together with a partial disappearance of the transverse planes and the mitochondrial granules. It may be that the mitochondrial substance has become spread upon or through the planes as under these conditions they take a deep stain.

The consideration of the majority of earlier views regarding the origin of the myofibril can be brief, since they are plainly based upon preparations showing but incompletely the structure first seen by MacCallum and described in the preceding paragraphs. It is not necessary to consider separately the work on heart and skeletal muscle or to distinguish between the bird and the mammal, since variations in the character of the myofibrils in all of these instances is but slight. Wagener ('80), Mtodowska ('08), and Krukiewicz ('09) claim that the myofibrils when first identified appear to a microscopic examination as structureless filaments. Eycleshymer ('04) came to a similar conclusion for the skeletal muscle of Necturus. Marceau ('02) is not certain whether they are segmented or not. Bardeen ('11) refers to them as having no definite cross striation. Since the development of the mitochondrial concept many have become convinced that in one form or another it is the precursor of the myofibril. Meves ('09), Duesberg ('10), Asai ('14, '15), believe that the myofibrils can be traced back to the mitochondrial rods or

'chondriokonten,' and Torraca ('14) finds a similar origin during their regeneration. Brück ('09), Godlewski ('02), and Rubaschkin ('10) derive the myofibril from granular mitochondria. Luna ('13) finds that granules appear first in skeletal muscle while in the heart the primitive condition is an unsegmented fibril. Altman in 1894, before the name mitochondria had been introduced, expressed a belief in the granular origin of the myofibril.

It is not difficult to understand how many observers came to believe that granules, rods, or filaments preceded the adult myofibril. Each of these structures can be found in the sarcoplasm if the technique does not bring out the hexahedra and granules in their totality. As already indicated, heavy short rods and structureless filaments appear when the hexahedral structure is not perfectly preserved. The fine granules described by Godlewski ('00) were in many instances strung along slender filaments. He saw in part both elements of the sarcoplasmic structure. Schlater ('06) distinguished even the optical sections of entire hexahedra with their granules. He did not, however, observe them to be united in a continuous structure throughout the protoplasm.

It has already been said that MacCallum ('97), Wieman ('07), and Brück ('13) related either a structure of filaments or of planes to the development of the myofibril. In Wieman's ('07) valuable account for the chick he finds mitochondrial granules constantly at the intersections. MacCallum found them less frequently. The figures of the two do not show the predominance of four-sided optical sections or as much regularity in the size and arrangement of the spaces as was found in the preparations used in the present study.

A question of greater significance upon which the present account is at variance with their observations is the manner of appearance of the myofibril. Both authors believe it takes origin within rows of the spaces as a result of their subdivision into still smaller compartments. Wieman finds this process to be under way in the four-day chick. The measurement of the spaces in the heart at this stage by the writer with the aid of photographs did not furnish any evidence that they fell into two groups in

reference to their size. He has also expressed the belief that the contractile structure at this time does not consist of fibrils, but that it is a slight modification of the primitive hexahedral spaces with their mitochondril granules.

Brück ('13) described in the embryonic muscle cells of the lamellibranch Anodonta a primitive protoplasmic honeycomb which gives rise to fibrils by thickening at the intersection of the planes. Mitochondria is not only accumulated at the intersection of planes as in the higher forms, but is also present scattered along the developing fibril. Brück's observations are all the more valuable as a confirmation of the chief features of the accounts of MacCallum and Wieman, since one may conclude from his failure to mention their articles that he arrived at this view entirely independent of any suggestion from their work.

THE BEGINNING OF CONTRACTION

It has been said that rhythmic contraction is first to be seen in the seventeen- or possibly sixteen-somite chick. It is in accord with the usual history of developmental processes that there should be a preliminary contraction of a more simple kind leading up to it. If the claim had been substantiated that myofibrils take their origin at a definite period in development rather than by a gradual modification of a structure that has been in the heart from the first, there would have been some reason for anticipating that the contraction also would begin abruptly at the time of the appearance of the contractile structure. One writer placed the beginning of pulsation at a time after the appearance of the myofibril, another makes the two contemporaneous, and a third believes that the contraction comes first. None of them discuss a possible gradual assumption of the contractile function. Wieman has pointed out that there is no longer a question regarding the time of appearance of a myofibril, since it is a gradual differentiation of a structure present in the youngest heart cell. This consideration suggested to MacCallum that the contractile function also may be very gradual in its beginning.

A search was made for evidence of contraction in preparations fixed before the beginning of rhythmic pulsation. It was found

that in the heart of the ten-somite chick small areas of hexahedral structure often show elongation. They are of too limited extent to be explained as the result of stretching during the handling of the tissue. It is not impossible, then, that they are the expression of local muscle contractions. If this be true it is still a question whether the contraction occurred spontaneously or due to the stimulus of the fixing fluid.

Roux has described under the name 'fambrosia' a widespread response of various types of embryonic cells to unfavorable conditions which consist in their drawing away from each other. This can be seen in the early chick heart and is an undoubted expression of a protoplasmic contractility still more primitive than apparently occurs in the ten-somite heart.

SUMMARY

The heart of the chicks younger than the sixteen- or seventeen-somite stages, when rhythmical contraction begins, has a sarcoplasmic structure whose optical section is a net consisting of two systems of parallel lines intersecting to cut off spaces approaching a square form. They measure in the fixed material about 0.8 micron on a side. The apparent net is probably produced by three systems of membranes each parallel among themselves which intersect to form hexahedral compartments. At all intersections of three planes are small uniform mitochondrial granules.

There is some slight indication in the arrangement of the planes of a division of the early myocardium into mononuclear cell areas which correspond to lobations on its exterior. For a period including the thirteen-somite stage clefts appear partly separating cell areas. Yet at no time can cell walls be made out or any interruption of the planes crossing the region intermediate between two nuclei except these occasional clefts. The myocardium of the four-day chick shows no clefts. It was observed in tissue cultures to migrate out into the clot in the form of anastomosing multinuclear columns which gave no evidence of breaking up into cells other than slight constrictions of the columns. In

spite of this appearance of structural continuity throughout the sarcoplasm in the four-day chick, since Wieman has been able to divide it into mononuclear bodies by maceration and since temporary clefts appear in the myocardial tissue it is to be concluded there is probably some kind of demarcation into cell areas in the early myocardium.

The hexahedral structure of the pulsating heart through the fourth day of incubation differs from its earlier condition in the more rectilinear arrangement of its planes and in functional changes consisting in the elongation of the hexahedra over considerable areas in a common direction. This brings the granules of the planes transverse to the direction of elongation closer together so that they give the appearance of bars. The opposite phase to contraction is apparently in part due to an elongation of the hexahedra in a direction transverse to the original extension. The complete understanding of the functional changes in the form of the hexahedra was prevented by the inability to determine the extensions and elongations parallel to the optical axis of the microscope.

Several considerations suggest that the rhythmical beat is preceded by a less highly organized type of contraction. In the first place, the changes in the contractile structure through a period including the beginning pulsation and up to the fifth day are not very marked. Then also small areas in which the hexahedral structure is elongated in a common direction can sometimes be found in the heart of a ten-somite chick. It may be that these are the primitive local contractions brought about either spontaneously or through the stimulus of the fixing fluid. The creeping apart of embryonic cells described by Roux and observed in the early heart is evidence for the existence of a very primitive protoplasmic contractility.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
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PHAGOCYTOSIS OF CARBON AND CARMINE GRANULES IN THE TRANSPARENT TAILS OF TADPOLES

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ONE PLATE

The present study is part of a series dealing with the growth and reactive powers of the tissues and cells in the transparent tails of tadpoles toward external stimuli. These experiments were begun with the especial object of studying the behavior and growth-regulating factors of lymphatic endothelium, but necessarily included the observation of blood vessels, leucocytes, and mesenchyme cells. One of the authors ('09) had previously shown that the lymphatics react positively toward red blood cells extruded into the tissue, sending out new sprouts which grow toward them and actively engulf them. Also, the authors ('17) found that lymphatic capillaries will react positively toward fatty substances introduced into the subcutaneous tissue, growing toward the injected oil globules, and, in this case with the aid of leucocytes, taking part in their absorption. It is well known that lymphatics play an important part in the disposal of coal pigment, in anthracotic lung tissue, and in the absorption of foreign particles introduced into the peritoneal cavity and, for this reason, we thought it might prove profitable to test the response of lymphatics, in the transparent tails of tadpoles, toward injected granules of carbon and carmine.

The experiment of injecting small quantities of India ink into the tissue spaces of the tail fins of tadpoles was first carried out in 1913 in the course of other studies on growing lymphatics. It was found that the carbon granules were taken up by leucocytes and by mesenchyme cells, but that lymphatics and blood vessels

were apparently indifferent to the presence of foreign particles of this nature in their vicinity.

Although the presence of carbon granules in the tissue spaces did not prove to be a stimulus for the growth of lymphatics, it seemed advisable to record briefly the reaction of the various cells and tissues toward this substance and especially that of the mesenchyme cells, in view of the fact that the phagocytic powers of these cells has been disregarded or denied by most investigators of the subject. The region of study—the transparent tail of the tadpole—where each individual cell can be watched, recorded, and followed for days, or weeks if necessary, and the method of chloretone anesthesia and observation in the upright chamber which permit of study in vivo under approximately normal conditions, both possess marked advantages over many of the regions and methods selected by others for the study of phagocytosis.

The tadpoles used for these experiments were the larvae of Bombinator, Rana pipiens, and Hyla pickeringii. The method of introducing small amounts of foreign substances was the same as that described previously for injections of paraffin oil (E. R. Clark, '16) and fat (E. R. and E. L. Clark, '17). The injection materials—India ink, diluted one-half with tap water, and powdered carmine, suspended in distilled water—were placed in small vials, which were set in a dish of boiling water for half an hour. The tadpoles were anesthetized in chloretone, 1 to 3000, and small amounts of the ink or carmine were injected into both tail fins, through fine glass cannulae, under the binocular microscope. These suspensions of granules were injected at different points in the fins—in some cases near the margin and, on a few occasions, they were injected directly into the lumen of a lymphatic capillary. The tadpoles were then transferred to the observation chamber, devised and described by one of the authors ('09, '12) and studied under the compound microscope in a 1 to 5000 solution of chloretone. Records of the site of injection, including all the cells and vessels in the neighborhood, were made soon after the injection with the aid of the Leitz drawing eye-piece. The tadpoles were then returned to fresh water.

The injected regions were observed on succeeding days, records were made of the same cells and vessels and of the changes which occurred.

The results obtained from injecting carbon, in the form of India ink, and carmine granules proved to be identical, and a single description will suffice for them both.

Leucocytes were the first cells to respond to the presence of the carbon and carmine granules. About an hour after the injection some of these cells, containing red or black granules, could be seen near the injection site. For several days after the injection, leucocytes continued to migrate toward the point of injection and to take up the injected granules. These cells became loaded with the carbon or carmine to such an extent, in some instances, that they resembled merely balls of pigment with no cellular matter visible. Many of the leucocytes, after taking up the ink or carmine, moved over to a near-by blood vessel and flattened out on its wall. In some cases such a pigmented leucocyte was observed to crawl through the vessel wall. But the removal of foreign particles by this method was evidently very slow, since many cells, loaded with the granules, remained close to the exterior wall of blood capillaries for long periods before entering the vessels. Other pigmented leucocytes did not migrate to blood vessels or lymphatics, but instead they wandered away through the tissue spaces of the tail fin. In the case of cells which had taken up carbon granules, this process was difficult to follow, owing to the fact that wandering cells containing black pigment are usually present in the tadpole's tail, under normal conditions, but it was easy to trace the wanderings of the leucocytes containing carmine, and these cells were found scattered through the tail fin at long distances from the site of injection.

Records made ten days and two weeks after injection, showed collections of leucocytes still present near the site of injection, all of them filled with black or red pigment and most of them adherent to the walls of blood vessels. By this time the number of leucocytes present in this region had diminished somewhat. Since the phagocytosis of foreign particles by wandering cells

and leucocytes is such a well-known phenomenon, it seemed superfluous to investigate the character of these cells, in the tadpole's tail, and their response toward the injected substances in anything but an incidental manner.

The response of the mesenchyme cells—and by this term we mean the stellate connective-tissue cells—was much slower than that of the leucocytes and wandering cells, and for several hours after an injection no reaction was noted. On the day following the injection, an occasional granule of carbon or carmine could be detected along the processes of some of these connective-tissue cells. From this time on the number of granules taken up by the cell processes of the mesenchyme cells increased and, after three or four days, clumps of granules were visible in the cell bodies near the base of the processes as well as on the processes.

After ten days or two weeks the number of mesenchyme cells containing red or black granules and the amount of foreign pigment present in these cells had both increased. This increase occurred at a time when the number of mobile phagocytes had begun to diminish. The carbon or carmine persisted within the mesenchyme cells, at the site of injection, for as long as the tadpoles remained under observation.

One of the authors ('12) has made a careful study of the movement of these so-called 'fixed' connective-tissue cells. He found that their shape is continually changing: processes are withdrawn on one side and sent out on the other, and that by this means these cells are capable of a slow amoeboid locomotion. The same kind of movement was noted in the records made of these cells before and after taking up the foreign particles, but in comparison with the behavior of the leucocytes this migration of the mesenchyme cells is quite negligible. The granules inside of leucocytes were conveyed to blood vessels and eventually transported by the blood stream to other portions of the body or they were carried through the tissue spaces of the tail to a relatively great distance from the site of injection. On the other hand, those carmine and carbon granules whose fate it was to be picked up by mesenchyme cells remained relatively stationary, near the spot at which they had been originally introduced into the tail.

Figure 1 is a drawing made two weeks after an injection of a suspension of carmine granules. The number of leucocytes in this region has diminished by this time and several wandering cells containing red pigment can be found scattered through the fin at some distance from the point of injection. Those that remain for the most part have congregated near the wall of a near-by blood capillary and two of them are shown in the act of entering the vessel. Almost all of the mesenchyme cells of the region contain carmine granules. Figure 2 is a high-power drawing of such a mesenchyme cell, selected from a similar specimen, which shows the arrangement of phagocytized carmine granules. As may be seen, these red granules have collected on the processes and in the cell body near the base of the processes. Figure 3 is a drawing of a number of mesenchyme cells made five days after an injection of India ink into the subcutaneous tissue of the tail fin and shows an identical response on the part of this type of cell toward the granules of carbon which have collected, in a similar manner, on the branched processes and within the cell bodies near the base of the processes.

It is evident, therefore, that in a transparent region such as the tadpole's tail where each individual cell may be observed in detail, in the living, and under practically normal conditions and where the different cell types may be distinguished with ease, that the mesenchyme cells are active phagocytes of foreign particles, such as carbon and carmine, present in the tissue spaces. Their reaction toward these foreign substances is less rapid and intense than that of the leucocytes, but it is nevertheless definite and probably important. The present observations gave the impression that granules housed within the mesenchyme cells were more permanently located than those which had been engulfed by leucocytes, since that portion of the foreign pigment remaining at the point of injection two or three weeks after it had been introduced is, for the most part, retained in the interior of connective-tissue cells.

We have made the statement that the lymphatics of the tadpole's tail showed no visible reaction toward these foreign particles present in the tissue spaces. The sending out of new

sprouts which grew toward the injected granules—the characteristic response of the lymphatics toward extruded blood cells and injected fat globules—was never observed in the case of the injections of carbon and carmine granules. However, in repeating these experiments during the past year, we found that the lymphatic endothelium, although not stimulated to grow toward these foreign substances when they were at a distance, would react toward them when they were in very close proximity. On a few occasions, a small amount of India ink was injected directly into the lumen of a lymphatic capillary. When such a tadpole was examined on the following day, carbon granules were found to be enclosed within the endothelial cells of that lymphatic which had been injected with ink. They were present as small black spots and as large clumps of granules within the areas surrounding the nuclei of the endothelial cells—the same region which stains with vital dyes (E. R. Clark, '09; Wislocki, '16).

A similar behavior on the part of blood-vessel endothelium was not noted.

That foreign particles, such as carbon, carmine, cinnabar, etc., introduced into the blood stream or subcutaneous tissue are quickly phagocytized within the body has been known for many years. Thus, Ponfick ('69) and Siebel ('89) showed that such substances as cinnabar and indigo injected into the blood stream quickly disappeared from the circulation and collected in the liver, spleen, and bone marrow, where they were taken up by cells with large round nuclei, resembling leucocytes.

Metchnikoff ('83), in his studies on inflammation, described the phagocytosis of carmine granules and of red blood cells injected into Triton larvae and into the tails of tadpoles, by leucocytes which have migrated from the blood stream and by wandering cells of the tissues. Later ('92), in describing further observations, he groups these two types of cells together as macrophages or cells whose chief function is the phagocytosis of foreign particles and of cell débris.

Muscatello ('95) and MacCallum ('03) found that carmine and carbon granules, injected into the peritoneal cavity, were conveyed to the lymphatics of the diaphragm by means of leuco-

cytes which collected in the peritoneal cavity in large numbers and actively engulfed the foreign particles. These results differed from the conclusion of Von Recklinghausen that such particles reached the lymph vessels in the free state through large openings, or stomata, in the endothelial wall.

Recent studies on anthracosis of the lungs made by Haythorn ('13) and Klotz ('14) abandon the older view that the coal pigment is taken up by the epithelial cells of the alveoli and that it frequently reaches the lymph glands draining the lung in the free state, cutting through the walls of the lymph vessels by means of the sharp corners on the granules. These authors find that all the coal pigment in anthracotic lung tissue is present inside of phagocytes, which, according to Haythorn, are probably 'endothelial' leucocytes, which pick up the coal pigment in the alveoli, convey it to the lymphatics and lymph nodes and also to the connective-tissue septa. They state that the pigment remains indefinitely intracellular.

The origin of these large mononuclear wandering cells and leucocytes is still undecided, but their importance as phagocytes of foreign particles and cell débris is unquestioned.

The phagocytic power of the specialized endothelium in certain regions of the body has been emphasized by many authors. The endothelial cells of the liver and bone marrow and the reticulo-endothelial cells of the spleen and lymph glands have long been recognized as phagocytes of foreign material, and Evans ('15) has grouped this type of phagocyte with the large mononuclear wandering cells and the clasmatocytes as macrophages. Mallory ('14) even takes the extreme view that the whole group of mononuclear wandering cells and leucocytes, which are phagocytes of foreign particles and cell débris are derived from the endothelium.

MacCallum ('03) found that the endothelial cells lining the lymph vessels of the diaphragm were packed with carbon granules after injections of India ink into the peritoneal cavity. The observation of one of the authors (E. R. Clark, '09), on the taking up of red blood cells from the tissue spaces, showed that the growing lymphatics of amphibian larvae are phagocytic. In

this instance the foreign substance, in the form of red blood cells, was taken into the lumen of the lymph vessel. The observation of Wislocki ('16), that the colloidal dye trypan blue is stored in the form of granules in the perinuclear areas of the lymphatic endothelium of tadpoles, renders it highly probable that all the endothelial cells of the lymphatics in amphibian larvae possess the power of phagocytosis. This conclusion of Wislocki's is based on the work of Evans and Schulemann ('14, '15) which appears to prove that the storage of acid azo dyes, in the form of aggregations of dye granules, is evidence of phagocytosis on the part of cells which display this behavior. The present observations on the fate of carbon and carmine granules introduced into the lumen of lymphatic capillaries demonstrates this phagocytic power of the lymphate endothelium, for these granules were taken up by the endothelial cells lining the lymphatics and stored in the perinuclear areas.

The phagocytic power of the mesenchyme cells has not been so generally recognized in the literature. The ability of these cells to ingest foreign material was not studied in detail by the earlier writers, while recent investigators frequently have doubted or denied its existence. That deposits of foreign pigment, such as coal pigment in anthracosis, metallic silver in cases of argyria, etc., are present in the connective tissues has long been known, but recent authorities claim that such material is stored solely in the resting wandering cells or in mononuclear leucocytes or in endothelial cells, such as the Kupffer cells of the liver, and not in the connective-tissue cells proper, or fibroblasts. MacCallum ('03) is an exception to this statement, for in his description of the results of injecting India ink into the peritoneal cavity, he mentions the presence of carbon granules in true connective-tissue cells.

Haythorn ('13), in his studies on the histology of anthracosis, describes wandering cells or endothelial leucocytes as the only cells which phagocytize the coal pigment. Carbon granules present in the connective-tissue septa he believes to have been conveyed there by the mobile phagocytes and to remain permanently within these cells. Metchnikoff ('83), in studying the

effects of injecting carmine granules into the transparent tails of Triton larvae and tadpoles, notes and figures these foreign granules inside of mesenchyme cells as well as in the wandering cells and leucocytes. However, in later studies ('92) he recedes from the opinion that the connective-tissue cells are phagocytic and states that mesenchyme cells which contain foreign pigment are cells which have ingested it while they were leucocytes and that leucocytes containing ingested pigment may wander off and become transformed into cells which resemble mesenchyme cells. In a still later publication ('05) he denies the power of true mesenchyme cells or fibroblasts to phagocytize foreign matter. Similarly, Buxton and Torrey ('06), in describing the disposal of carbon granules injected into the peritoneal cavity, note the rapid phagocytosis of these particles by the macrophages and consider that the carbon granules, wich are found several hours later in the connective tissue, have been conveyed there by these macrophages, which then proceed to transform themselves into 'trailers.' However, the authors are not absolutely convinced that these 'trailers,' which are practically stationary and which store the granules indefinitely, are invariably transformed macrophages and they state that these cells are frequently indistinguishable from ordinary connective-tissue cells.

Recently, Jones and Rous ('17) have denied the phagocytic power of mesenchyme cells. Their rather elaborate method of study consisted in making suspensions of individual cells by digesting with trypsin the clot of proliferating tissue cultures of embryonic chick material. The individual cells were then freed from the tissue mass by filtering through gauze, and new cultures were then made to which finely ground carmine was added. A slight amount of phagocytosis occurred, but mainly on the part of large cells which the authors believed to be endothelial in nature. The authors concluded that the connective-tissue cells proper possess no power of phagocytosis.[1]

[1] Since writing this article, the recent work of Leo Loeb and Fleisher ('17) has come to our attention. These authors, in studying the behavior of connective-tissue cells in tissue cultures, question the decision of Jones and Rous that the large cells which phagocytize foreign particles, in their tissue cultures are endo-

In the present experiments, with a region of study such as the transparent tail fin of Amphibian larvae and with the aid of chloretone anaesthesia and the special observation chamber, it was possible to see and to keep records of the individual cells of the area at the time of the injections of India ink and of carmine granules, and to follow these cells and to observe their subsequent behavior with perfect clearness and in their normal environment. When the living cells are observed in this manner, it is perfectly clear that mesenchyme cells possess the power of phagocytizing foreign particles of carbon and carmine and of retaining them indefinitely. It is very easy, by making records of individual mesenchyme cells, to see that these same cells which were present as connective-tissue cells at the time of injection later contain granules of carbon or carmine and that they retain their characteristic identity as 'star-shaped' cells after taking up the pigment. Also it is clear that the wandering cells and leucocytes which ingest the foreign particles do not become transformed into connective-tissue cells, even in the cases where they wander off through the tissue spaces, but that they persist as round or amoeboid cells, easily distinguishable from the branching connective-tissue cells.

CONCLUSION

The present observations show that three types of cell present in the transparent tails of tadpoles display the power of phagocytizing granules of carbon and carmine injected into the subcutaneous tissue. These are: leucocytes (including wandering cells), stellate connective tissue-cells, and the endothelial cells of the lymphatics. The leucocytes actively migrate toward the site of injection, while the mesenchyme cells and lymphatic endothelial cells apparently ingest only those particles which are in close proximity to them.

thelial in nature. Loeb and Fleisher consider such cells to be fibroblasts which have increased in size during the process of regeneration and which are capable of ingesting foreign particles and cell débris.

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PLATE 1

EXPLANATION OF FIGURES

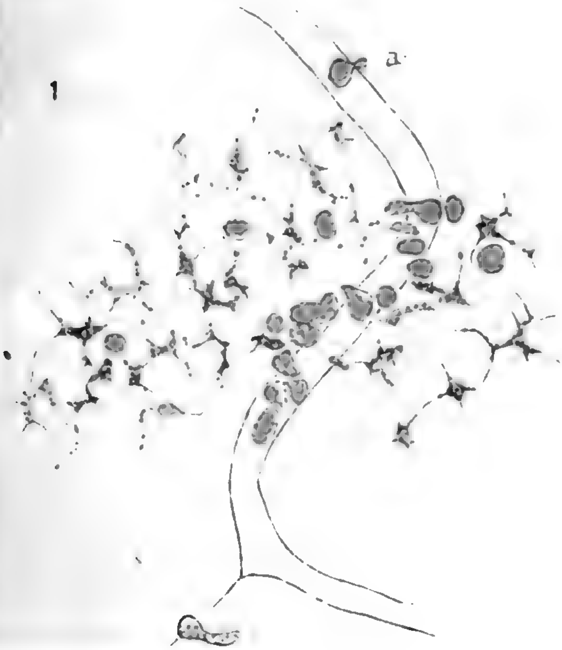
Fig. 1 Camera-lucida drawing of a region in the dorsal fin of a tadpole, into which a suspension of carmine granules had been injected two weeks previously. The sketch shows that the red granules have been taken up by leucocytes and connective-tissue cells. *a*, Leucocyte, containing carmine, on the point of entering a blood vessel. Enlargement = × 275.

Fig. 2 High-power drawing of a single mesenchyme cell, showing phagocytized carmine granules, distributed on the cell processes and in the body of the cell, near the base of the processes.

Fig. 3 Camera-lucida drawing of a number of mesenchyme cells from a region of the tail fin into which India ink had been injected five days previously. Carbon granules have been taken up by the connective-tissue cells and are lodged on the processes of the cells and in the cell bodies near the base of the processes. Enlargement = × 300.

1

a



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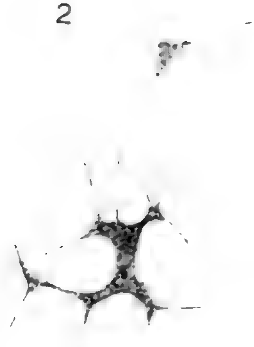

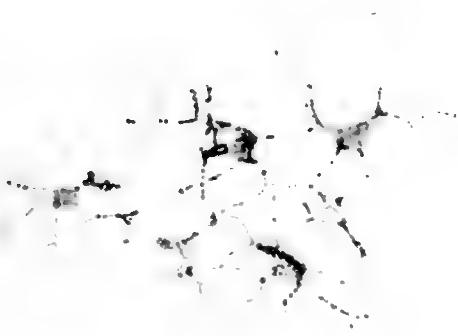

3

ON THE TIME OF THE POST-NATAL OBLITERATION OF THE FETAL BLOOD-PASSAGES (FORAMEN OVALE, DUCTUS ARTERIOSUS, DUCTUS VENOSUS)

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THREE FIGURES

It is generally recognized that two separate processes are involved in the occlusion of the fetal blood passages after birth. The first is the simple functional closure which takes place, in the great majority of cases, at or immediately following birth; the second is the permanent anatomic obliteration which occurs at a later period. The mechanics and histology of the latter process are generally discussed in our larger treatises on obstetrics and pediatrics, and in our major anatomical texts; but the time of postnatal obliteration is often unmentioned, although in a number the statement is made that the obliteration takes place in the first few days or, at most, weeks after birth.

The origin of the current concepts as expressed in these larger texts can be traced, we think, to the first statistical study on this subject, which was published ninety years ago by the French clinician Billard. This investigator collected data on the obliteration of the ductus venosus, ductus arteriosus, and foramen ovale in a series of one hundred and twenty-eight children who died in the first eight days of life. He found instances of the obliteration of all these passages on the first day after birth. The foramen ovale and ductus arteriosus were closed in over fifty per cent of his cases on the eighth day while the ductus venosus was closed in a still larger number. He therefore concluded that the obliteration of the fetal blood-passages proceeded very rapidly in the first few days of life—an opinion in accord with that

THE ANATOMICAL RECORD, VOL. 15, NO. 4
NOVEMBER, 1918

held by a number of writers in the eighteenth century. The results of Billard's study were published in his 'Traité des maladies des enfants nouveau-nés' in 1828. This work was extremely popular in its time; it passed through a number of French editions, was translated into Italian and German and twice appeared in American editions. In several publications of the middle of the last century Billard's figures are cited and his name is quoted in connection with them. In later works, however, the same opinion has been repeatedly expressed but its source apparently has been forgotten.

A large amount of data concerning the chronology of the postnatal obliteration of the fetal blood-passages has accumulated since the time of Billard. This material is scattered through the periodical literature of legal medicine, obstetrics, and pediatrics and is also included in a number of rather inaccessible brochures. In the following pages we have collected as much as possible of these scattered data and have arranged them in tabular and graphic form. In so doing we have confined ourselves to those records in which series of consecutive cases have been assembled. These records include observations on children who were born at term, and also a few cases of children who were prematurely born. In most instances, however, investigators have failed to separate these two groups. A comparison of the records of the few known cases of prematurity with those of children known to be born at term shows no appreciable difference in the time of obliteration of the fetal blood-passages. We have therefore combined them in our tables. In all cases the patency of the fetal passages was tested either by injection or by probing, excepting those of Faber ('09) which were examined microscopically.

The results made apparent by this combined series of observations are at variance with the current concepts on the subject as expressed in most of our larger texts, and also with the results of Billard. Billard's observations were confirmed by Bernutz ('65) who found the ductus arteriosus closed in fourteen cases in a series of twenty-one children who died between the tenth and twentieth days, and in thirty-six out of thirty-eight children dying between the twentieth and sixtieth days. Since this time

no observer has substantiated these findings, although a number of series much larger than those of Billard and Bernutz[1] have been collected. Thus Elsässer ('52), in a series of nearly three hundred observations upon children of the first month, found obliteration of the ductus arteriosus in about two per cent and of the foramen ovale in about three per cent of his cases; and Alvarenga ('69) found practically no instances of obliteration of the foramen ovale or ductus arteriosus before sixty days. The findings of later observers (Alexeieff ('00), Theremin ('87–'95), Kucheff ('01) and others) agree essentially with those of Elsässer and Alvarenga although they have noted some instances of earlier obliteration of the passages.[2]

THE FORAMEN OVALE

The compiled data on the obliteration of the foramen ovale are shown in extenso in table 1. Table 2 is a summary of these data giving by periods the total number of observations and the number and per cent of cases obliterated. Graphic representations of these data are shown in figure 1 curve A, and in figure 2. In the summary and curve the data of Billard are omitted because his findings are so directly opposed to those of all other investigators that we conclude that either his method of investigation was defective or that his definition of obliteration was entirely different.

As will be seen in table 2, less than one per cent of the foramina are completely closed in the first week of life, and less than two and one-half per cent in the second week. In the latter part of the first month the figures indicate that the obliterative process takes place more rapidly as the opening is impervious in about one-eighth of all cases of this period. The rapidity of this process increases during the second month, for the interatrial

[1] The cases reported by Bernutz were not examined by him personally but were collected at his instance by the interne of a colleague in the Hospice des Enfants-Trouvés.

[2] Haberda ('96) studied the obliteration of the ductus venosus and ductus arteriosus in a considerable series of infants and children. As his data are not given in numerical form we are unable to include them in our summary. The general statements of this writer indicate that his findings were somewhat similar, as regards these two vessels, to our own.

TABLE 1

Data on the obliteration of the Foramen ovale. Numerals enclosed in parentheses indicate number of obliterated cases

Observer and date	0 to 8 days	8 to 15 days	15 to 22 days	22 to 32 days	32 to 46 days	46 to 61 days	61 to 91 days	3 to 6 months	6 to 9 months	9 to 12 months	1 to 5 years	5 to 10 years	10 to 20 years	20 years and over
Alexeieff, '00.	3	7	6	11	21	25 (1)	46 (3)	68 (6)	15 (3)	3	2 (2)	1 (1)	1 (1)	
Alvarenga, '67	44	19	17	33	28	12	23 (2)	18 (1)	1	4	12 (4)		2 (1)	
Billard, '28...	118 (17)	20 (11)												
British Anat. Soc., '98...											23 (11)	9 (7)	30 (23)	286 (217)
Bizot, '37....												* 34 (23)		63 (49)
Elsässer, '52.	150 (1)	63 (4)	62 (9)	23 (2)	3 (1)									
Fawcett......													6 (3)	299 (207)
Letourneau, '58	8 (1)	1												
Ogle, '57.....														62 (49)
Theremin '87	70	76	63 (10)	49 (13)	55 (17)	45 (18)	81 (47)	65 (51)	11 (9)	7 (7)				
Theremin '95	18	5	4	6 (1)		4 (2)	8 (3)	6 (6)	† 6 (6)		4 (4)			
Wallmann, '59											2	2	4	291 (170)
Zahn.........														711 (552)
Totals.....	293 (2)	171 (4)	152 (19)	121 (16)	107 (18)	86 (21)	158 (55)	157 (64)	27 (12)	20 (11)	43 (21)	12 (8)	42 (28)	1712 (1228)

* 1 to 15 years
† 7 to 10 months.

TABLE 2

Obliteration of the Foramen ovale (3258 cases)

AGE	TOTAL NUMBER OF CASES	NUMBER OF CASES OBLITERATED	PER CENT OF CASES OBLITERATED
Birth to 8 days	293	2	0.7
8 to 15 days	171	4	2.3
15 to 32 days	273	35	12.8
32 to 46 days	107	18	16.8
46 to 61 days	86	21	24.8
61 to 91 days	158	55	34.8
3 to 6 months	157	64	40.7
6 to 9 months	27	12	44.4
9 to 12 months	20	11	55.0
1 to 5 years	43	21	50.0
1 to 15 years	93	52	55.9
5 to 10 years	12	8	66.6
10 to 20 years	42	28	66.6
15 years and over	1840	1320	71.7
20 years and over	1712	1228	71.7

communication is obliterated in approximately one-sixth of all cases in the first half of this period and in about one-fourth in the latter half. During the third month somewhat less than ten per cent of the cases are obliterated so that by the end of the first trimester the foramen ovale is finally closed in about one-third of all cases. After the end of the third month the process again goes on more slowly and the average of obliteration in the second trimester is about forty per cent, that in the third trimester about forty-five per cent and that in the fourth trimester about fifty-five per cent. Our figures for the period between one and five years show an average obliteration of fifty-five per cent, which is five per cent less than that of the last trimester of the first year. This difference is due, no doubt, to the small number of cases tabulated in these two periods, and does not represent a real increase in the number of patent foramina. The figures of the second five year interval indicate that two-thirds of the foramina are closed; but the small amount of data for the period between the first and tenth years makes it impossible to say with certainty just when this increase in obliteration

is brought about. In the second decade the percentage of open foramina is the same as in the period between five and ten years and the foramen ovale is found to be obliterated in about seventy-two per cent of individuals of twenty years and over.

Curve A of figure 1 expresses graphically the frequency of obliteration of the foramen ovale during different periods in the first year of life. This curve is readily divisible into three parts. The first portion, which extends from birth to the middle of the

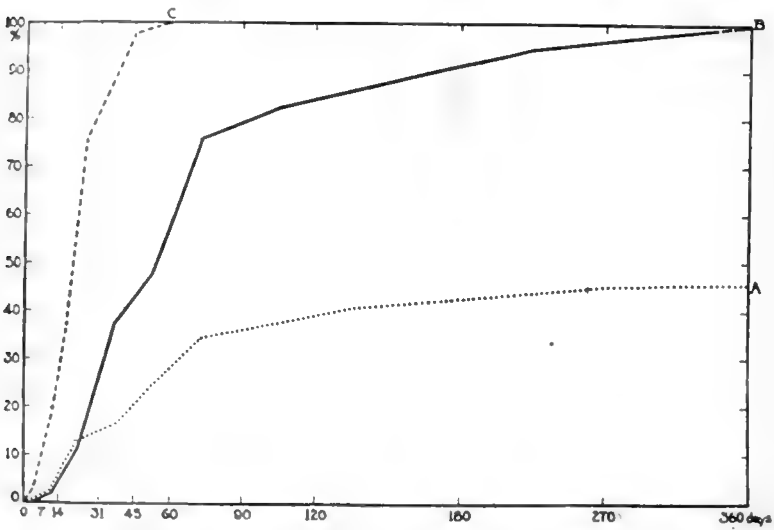

Fig. 1 Three curves representing the average percentages of obliterated fetal blood-passages at different periods in the first year of life. *A*, dotted line, foramen ovale, *B*, solid line, ductus arteriosus; *C*, broken line, ductus venosus. These curves are based upon the material summarized in tables 2, 4 and 5.

second week, is a short segment expressing the obliteration of a little over two per cent of the cases. It is followed by a longer segment rising abruptly and terminating in about the middle of the third month. Nearly half of the cases which are finally closed are obliterated in the period represented by this segment (sixty days). The third segment, extending from about the middle of the third month to the end of the year, shows a very slow but continuous rise and expresses the obliteration of about ten per cent of the total number of cases.

Figure 2 is a curve illustrating the frequency of the obliteration of the foramen ovale throughout life. The details of the obliteration during the first year, which have just been described, are masked in this figure by the diminution of the time unit. Here again three periods can be recognized which correspond roughly to infancy, childhood, and adolescence and maturity.

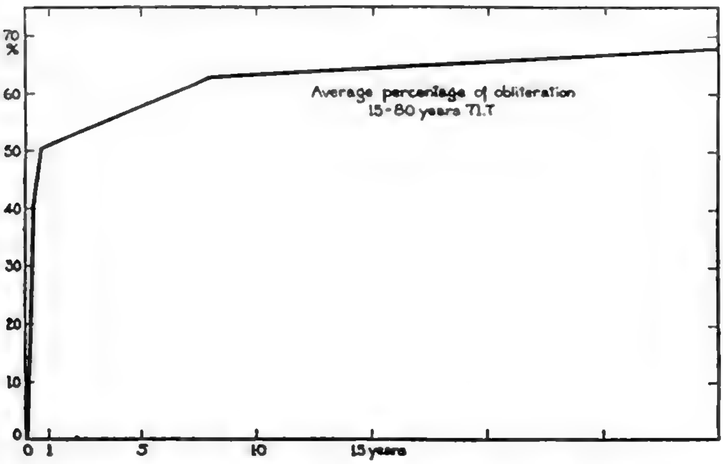

Fig. 2 A curve showing the average percentages of cases of obliterated foramen ovale at different periods throughout life. Based upon the material summarized in table 2.

During the first period the curve rises with extreme abruptness to a point at which fifty per cent of the cases are obliterated. The rise is continued but is much less rapid during the second period which extends from infancy well into childhood. In the third period, which extends to extreme old age, the curve rises very slowly to about seventy-two per cent. In all probability this final percentage of obliteration is reached in early maturity although the character of our data does not permit a graphic representation of this point.

THE DUCTUS ARTERIOSUS

Table 3 shows the compiled data upon the post-natal obliteration of the ductus arteriosus, and table 4 summarizes these data by periods. The graphic presentation of this material is shown

TABLE 3

Data on the frequency of post-natal obliteration of the Ductus arteriosus. Numerals enclosed in parentheses indicate number of obliterated cases

<table>
<tr><th>OBSERVER AND DATE</th><th>0 TO 8 DAYS</th><th>8 TO 15 DAYS</th><th>15 TO 22 DAYS</th><th>22 TO 32 DAYS</th><th>32 TO 46 DAYS</th><th>46 TO 61 DAYS</th><th>61 TO 91 DAYS</th><th>3 TO 4 MONTHS</th><th>4 TO 12 MONTHS</th><th>1 TO 10 YEARS</th></tr>
<tr><td>Alverenga, '69</td><td>19</td><td>11</td><td>15</td><td>28 (1)</td><td>17</td><td>8 (1)</td><td>14 (5)</td><td>9 (3)</td><td>1</td><td>7 (7)</td></tr>
<tr><td>Bernutz, '65</td><td></td><td colspan="2">* 21 (14)</td><td colspan="3">** 38 (36)</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Billard, '28</td><td>118 (16)</td><td>20 (11)</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Elsässer, '52</td><td>150 (1)</td><td>63 (3)</td><td>61 (6)</td><td>23 (1)</td><td>3</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Faber, '12</td><td colspan="4">° 5</td><td>3</td><td></td><td colspan="2">15 (14)</td><td>6 (6)</td><td>23 (18)</td></tr>
<tr><td>Gérard, '00</td><td>52</td><td>2</td><td>2 (2)</td><td>4</td><td>2 (2)</td><td>2 (2)</td><td>2 (2)</td><td></td><td>16 (12)</td><td>13 (13)</td></tr>
<tr><td>Kucheff, '01</td><td></td><td></td><td></td><td></td><td colspan="2">† 40 (18)</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Letourneau, '58</td><td>12</td><td>1</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Theremin, '87</td><td>67</td><td>66</td><td>61 (6)</td><td>56 (8)</td><td>50 (26)</td><td>43 (23)</td><td>68 (55)</td><td>53 (48)</td><td>54 (54)</td><td></td></tr>
<tr><td>Theremin '95</td><td>11</td><td>5</td><td>4 (2)</td><td>6 (3)</td><td></td><td>4 (1)</td><td>8 (8)</td><td>1 (1)</td><td>12 (12)</td><td>4 (4)</td></tr>
<tr><td>Totals</td><td>429 (17)</td><td>168 (14)</td><td>143 (18)</td><td>117 (13)</td><td>75 (28)</td><td>57 (27)</td><td>92 (70)</td><td>63 (52)</td><td>89 (84)</td><td>47 (33)</td></tr>
</table>

* 10 to 20 days.

** 21 to 60 days.

† 2nd month (data for other periods not available).

° 0 to 30 days.

TABLE 4

Obliteration of the Ductus arteriosus (1095 cases)

AGE	TOTAL NUMBER OF CASES	NUMBER OF CASES OBLITERATED	PER CENT OF CASES OBLITERATED
Birth to 8 days	311	1	0.3
8 to 15 days	148	3	2.0
15 to 22 days	143	16	11.2
22 to 32 days	117	13	11.1
32 to 46 days	75	28	37.3
46 to 61 days	57	27	47.4
61 to 91 days	92	70	76.0
91 to 120 days	63	52	82.5
120 to 365 days	89	84	94.5

in figure 1, curve B. As in the case of the foramen ovale, and for the same reasons, we have omitted Billard's data and also those of Bernutz from the summarized table and from the curve.

During the first week of life the percentage of obliteration of the ductus arteriosus is even less than that of the foramen ovale (three-tenths of one per cent). In the second week the percentage rises to an average of two and in the third and fourth weeks to an average of a little over eleven. From this time on to the end of the third month the process of obliteration is extremely rapid; in the first part of the second month it averages over thirty-seven per cent, in the latter part over forty-seven, and in the third month seventy-six per cent. During the fourth month the average obliteration is eighty-two per cent. Thereafter the percentage increases quite slowly until the end of the year. The average percentage of obliteration in the last three-quarters of the first year is nearly ninety-five.

The data available for the period after one year are small in amount and, with the exception of certain instances in Faber's series, all cases of this period were obliterated. It is quite possible that Faber's material included several specimens which, while containing remnants of the original lumen, were obliterated at other points. His method of examination might easily classify such cases as patent. It is probable, therefore, that table 3 shows a much lower per cent of obliteration for this period than is actually the case.

Curve B of figure 1 is the graphic presentation of the data summarizes above. Like the curve for the foramen ovale already described and represented in the same figure, this graph is readily divisible into three portions. The first segment extends from birth to the middle of the second week and shows a terminal obliteration of two per cent of the cases. The second segment rises abruptly, crosses that of the foramen ovale, and terminated at about seventy-five per cent in the middle of the third month. The third segment, which extends from the middle of the third month to the end of the first year, shows a continuously decreasing rate of obliteration and terminates at nearly one hundred per cent. Probably all normal cases are closed shortly after the first year, although there are numerous records of individual cases of the anomalous persistence of the lumen of the ductus arteriosus in later life.[3]

THE DUCTUS VENOSUS

The material compiled upon the obliteration of the ductus venosus is shown in table 5 and is represented graphically in curve A of figure 1.

TABLE 5

Obliteration of the Ductus venosus (762 cases)

AGE	TOTAL NUMBER OF CASES	NUMBER OF CASES OBLITERATED	PER CENT OF CASES OBLITERATED
Birth to 8 days	211	6	2.3
8 to 15 days	150	27	18.0
15 to 22 days	169	64	37.5
22 to 32 days	103	78	75.7
Second month	37	36	97.3
Third month	20	20	100.0
Fourth, fifth, and sixth months	41	41	100.0
6 months to 1 year	19	19	100.0
1 year and over	12	12	100.0

[3] For the literature on the subject of persistent patency of the ductus arteriosus the reader is referred to the papers of Poynter ('16), Wells ('08) and Gerard ('00[2]).

The process of obliteration is much more rapid in the ductus venosus than in the other fetal passages. During the first week the average is two and three-tenths per cent, in the second week it is eighteen per cent, in the third thirty-seven and one-half per cent, and in the last ten days of the first month about seventy-six per cent. During the second month the percentage rises to nearly one hundred and thereafter all cases are obliterated.

The curve shown in figure 1, while much more abrupt than that of the foramen ovale and the ductus arteriosus, is of the same general character and shows three segments. The first segment corresponds to the first few days after birth, and terminates between two and three per cent. The second segment rises with extreme abruptness to ninety-seven per cent in the middle of the second month. The third segment, which is very short, rises gradually to a full hundred per cent by the end of this month.

THE RATE OF THE OBLITERATIVE PROCESS IN THE SEVERAL PASSAGES

In order to study the *activity* of the obliterative process in the various fetal blood-passages we have calculated from the graphs shown in figure 1 the average daily rate of obliteration for a series of periods in early life. This was done by determining from the graphs the initial and terminal percentages of obliteration for each given period. The initial percentage was then subtracted from the terminal one and the figure thus obtained divided by the number of days in the period. For example, in the case of the ductus arteriosus the percentage of obliteration at the beginning of the second month was fifty-seven and at the close was seventy-nine and one-half. Thus twenty-two and one-half per cent of all cases were obliterated in this period of thirty days and the average daily rate of obliteration was seventy-five hundredths per cent. Table 6 shows the results of these calculations for the three passages and the curves in figure 3 express them graphically.

It will be seen by the examination of these curves that they have certain characters in common. Each starts with a low rate

TABLE 6

Approximate rate of daily obliteration of the Ductus venosus, Ductus arteriosus, and Foramen ovale in early childhood

PERIOD	DUCTUS VENOSUS	DUCTUS ARTERIOSUS	FORAMEN OVALE
1st week	0.33	0.04	0.10
1 week to 1 month	3.00	0.92	0.60
2nd month	0.60	1.60	0.45
3rd month	0	0.75	0.40
3 to 6 months	0	0.07	0.07
6 to 12 months	0	0.04	0.04
1 to 5 years	0	0	0.005

of obliteration, rises rapidly to a peak or maximum, and then declines to the base-line which represents the cessation of obliterative activity. In all cases the portion of the curve representing the decline in activity is less abrupt than is the initial rise.

Considering the curves individually, it will be noted that in the case of the ductus venosus both the initial and maximal rate of obliteration is much greater than that of the other two passages and that consequently the entire obliterative process is completed much sooner. The curve expressing the rate of obliteration of the ductus arteriosus is very similar to that of the ductus venosus although the initial and maximal rates are lower and the apex of the curve falls at a later period. While the curve of the rate of obliteration of the foramen ovale shows the three common characters indicated in the preceding paragraph it is markedly different from the curves of the vessels. It rises less abruptly to a lower maximum rate of obliteration which is maintained with but little loss for a much longer period, so that the curve presents a plateau which is entirely absent from the curves of the vessels. Following this plateau, the curve falls at first rapidly and then gradually over a long interval to the base line.

The results of this study may be summarized as follows:

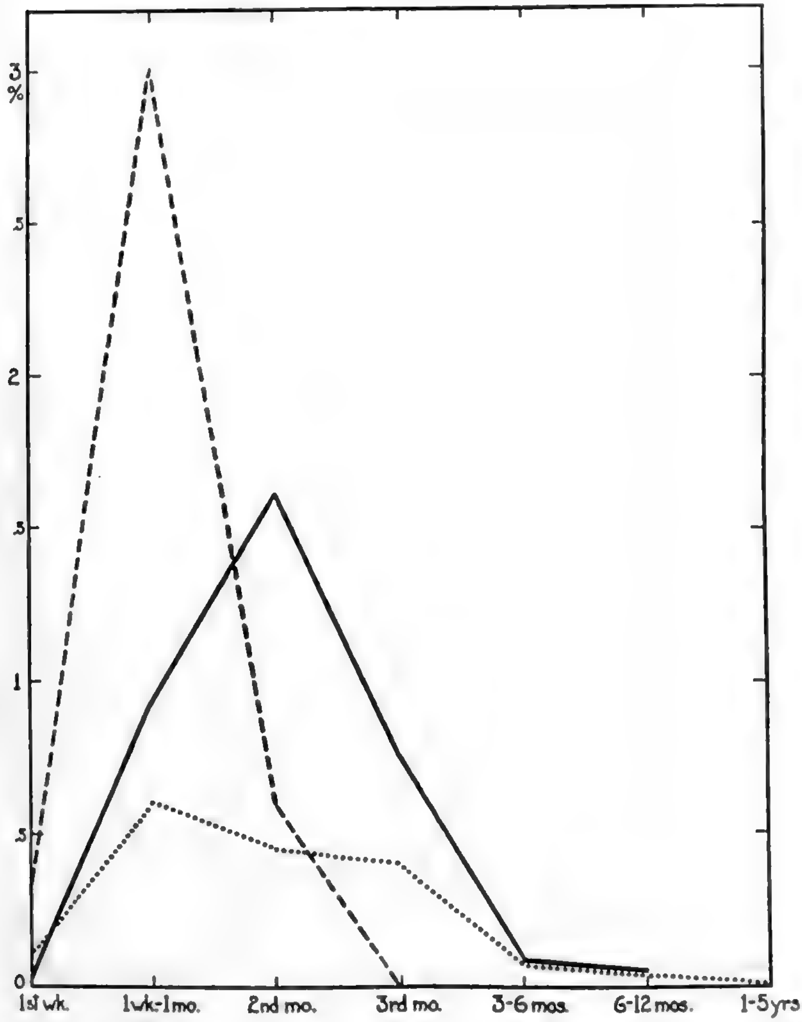

Fig. 3 Three curves showing the approximate average daily rate of obliteration of the fetal blood-passages in early life. *A*, dotted line, foramen ovale, *B*, solid line, ductus arteriosus; *C*, broken line, ductus venosus. Based upon the data summarized in table 6.

SUMMARY

The time of obliteration of the three fetal blood-passages (the ductus venosus, the ductus arteriosus, and the foramen ovale) is distinctly later than is commonly assumed. The process of obliteration in each of these passages shows three fairly distinct periods: an initial period with a low rate of obliteration, a middle period in which the rate of obliteration rises and the majority of cases are closed, and a terminal period in which the rate of obliteration is again slower.

Obliteration is most rapid in the ductus venosus. Although slow in the first week, the process reaches its maximum before the end of the first month and in the third month and thereafter all cases are closed.[4]

The ductus arteriosus closes more slowly. The obliterative process, which is very slow during the first two weeks of life, does not reach its maximum until the second month. Three-fourths of all cases are closed at the end of the first trimester and over ninety-five per cent by the end of the first year.

The period of the obliterative process of the foramen ovale is a matter of years rather than months. Beginning very slowly the process reaches its maximum activity near the close of the first month and continues with a slightly diminishing rate during the remainder of the first trimester. At the end of this time approximately one-third of all cases are closed. During the second trimester the rate of obliteration declines rapidly and thereafter decreases very slowly for an indefinite period—probably until early maturity, although few cases are closed after childhood. At the end of the first year about one-half of all cases are closed, in the second decennium about two-thirds, and in maturity about seventy-two per cent.

[4] It has been shown by the studies of Wertheimer ('86), Nikitin ('01), Fontan ('11) and others that the vein which sometimes occupies the center of the ligament of the ductus venosus in older children and adults is a new vessel developed after the obliteration of the ductus venosus and is not derived from the remains of this trunk.

POSTSCRIPT

After this paper was in press we secured a summary of one hundred and eighty-seven observations by Parrot on the obliteration of the ductus arteriosus. Parrot's findings are in general agreement with those of other observers which we have summarized above. Unfortunately his cases under one year are grouped in such a way that we cannot include them in our table 4, but if this were possible they would evidently affect our averages very little. Parrot found the ductus arteriosus patent in four cases in thirty-three of one year and in one in fifty-four of two years. In seventeen cases of three years and over the the ductus was always obliterated.

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AUTHORS' ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, OCTOBER 8

THE EARLY APPEARANCE OF THE ANLAGEN OF THE PARS TUBERALIS IN THE HYPOPHYSIS OF THE CHICK

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FIVE FIGURES

It is now well recognized that the epithelial portion of the hypophysis consists of three distinct parts. The pars anterior propria is the principal epithelial lobe and constitutes the main bulk of the gland, the pars intermedia is a thin layer, epithelial in nature, which becomes intimately associated with the neural lobe. The most recently recognized epithelial lobe is the pars tuberalis—so named by Tilney ('13) on account of its close relation to the tuber cinereum. It extends forward from the junction of the pars intermedia and the pars anterior propria, surrounding the infundibular stalk and spreading out for some distance under the brain floor.

The pars tuberalis has been sometimes confused with the pars intermedia—Lothringer ('86) and Herring ('08)—but recent studies have shown conclusively that these two parts are different both in adult structure and in developmental history. Tilney, in summarizing the development of the pars tuberalis in the chick and the cat, states:

> In addition to the histological differences between these two parts, the ontogenesis of the organ as observed in the cat and the fowl still further emphasizes the fact that the pars tuberalis and the pars infundibularis (pars intermedia) are morphologically distinct elements. The pars infundibularis makes its appearance immediately after the anlage of the buccal portion of the hypophysis is formed. The pars tuberalis arises as a relatively late structure. It has its origin in two secondary diverticula or sprouts from the body of the pituitary sac. These sprouts, the tuberal processes, ultimately fuse with each other across the median line, displace the body of the pituitary sac ventrad and thus secondarily assume their juxta-neural position.

One of us (Atwell ('18)) in a recent study of the development of the hypophysis in the rabbit has obtained somewhat different results. While agreeing with Tilney as to the distinctness of the pars tuberalis and the pars intermedia, and also confirming the statement that the pars tuberalis is late in acquiring its adult relationship with the tuber cinereum, it has been found that in the rabbit the anlagen of the pars tuberalis may be discerned very early. They were found to precede the definite pars intermedia by a considerable period of time.

It was with the hope of throwing some light upon this point that the present study was undertaken. Accordingly we have been led to construct a number of wax-plate models of the epithelial hypophysis from chick embryos, beginning with stages in which the tuberal processes might be recognized easily and then proceeding to successively younger embryos in an effort to determine the earliest appearance of the anlagen.

The literature relating to the lateral lobes and the pars tuberalis in the hypophysis of the chick is not extensive.

Rossi ('96) speaks of a median and two lateral parts in the early hypophysis of the chick embryo. According to Rossi the lateral lobes are secondary structures.

Economo ('99) observed a pair of 'Seitensprossen' in the hypophysis of the dove and of the domestic fowl. In dove embryos the sprouts appear between the fourth and seventh days of incubation. No definite statement is made concerning the first appearance of the sprouts in the chick.

Tilney ('13) first observed the 'tuberal processes' in a chick embryo of 5 days and 20 hours of incubation. From this stage the processes were traced to the formation of the pars tuberalis of the adult fowl. Although a reconstruction was prepared from an embryo of four days of incubation the tuberal processes were not seen in this stage. Embryos younger than four days were not studied.

Woerdeman ('14) notes that the lateral lobes are forming in a chick embryo of about 72 hours of development. The thickened epithelium which lies in front of Rathke's pocket is constricted off from the mouth cavity by two lateral folds. Woerdeman con-

siders that the lateral lobes so formed arise independently of Rathke's pocket.

Bruni ('15) observes the presence of two 'lobi laterali' in the chick at 82 hours of incubation. He also figures and describes the lateral lobes in older embryos but does not trace them into the formation of the pars tuberalis.

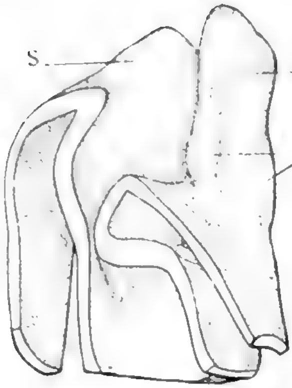
OBSERVATIONS

We have prepared wax-plate reconstructions of the epithelial portion of the hypophysis from chick embryos of 48, 59, 67, 72, 96, 120 and 144 hours of incubation. A relatively high magnification was chosen for the construction of the models in order that all details of structure might be shown as accurately as possible. For all younger stages, including the 72 hour embryo, the magnification was 300 diameters. For the older embryos the magnification was reduced to 200 diameters.

Chick embryo, 48 hours of incubation (21–2 pairs of primitive segments). Fig. 1. The hypophyseal pouch is well formed but opens widely into the mouth invagination. There is no indication of the lateral lobes. The anterior end of the fore-gut, which will later form Seessel's pouch, extends farther cranially than does the hypophyseal pouch. At this time the oral membrane is intact.

Chick embryo, 59 hours of incubation (30 pairs of segments). The hypophyseal pouch (Rathke's pocket) has deepened and now exhibits two lateral enlargements near its attachment to the oral epithelium. As later models show, these are the anlagen of the lateral lobes from which the tuberal processes develop. As may be seen from figure 2, Rathke's pocket is slightly constricted just above the lateral lobes. The lateral lobes have the form of blunt ridges which protrude laterally and also somewhat nasally. Their long axes lie parallel with the long axis of the entire hypophyseal pouch. This embryo shows one small perforation in the oral membrane.

Chick embryo, 67 hours of incubation. The lateral lobes are more prominent at this stage due to the fact that the hypophyseal pouch is beginning to be constricted somewhat from the



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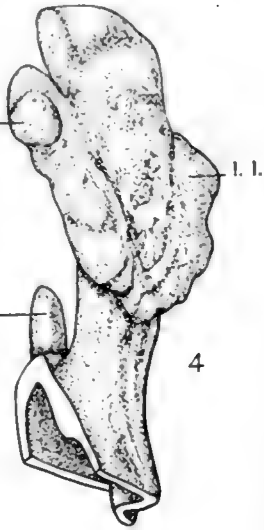

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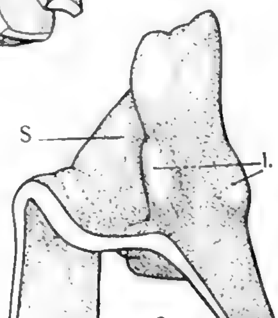

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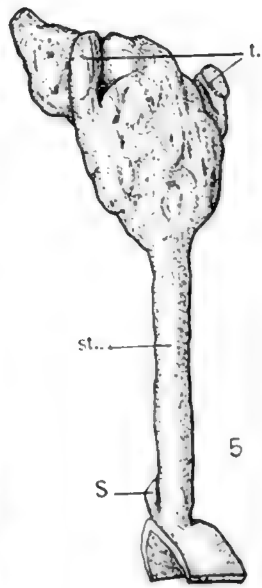

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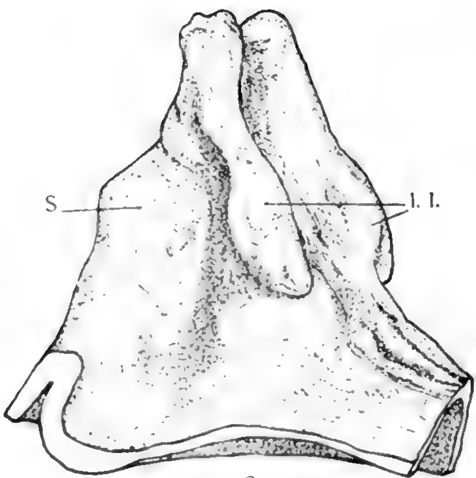

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oral cavity. The constriction of Rathke's pocket dorsal to the lateral lobes is also more distinct than previously. Each lateral lobe contains a lumen communicating with the cavity of the main hypophyseal sac. Seessel's pouch is in contact with the dorsal wall of Rathke's pocket for a considerable area. This is the ecto-entodermal fusion which has been recorded by numerous observers.

Chick embryo, 72 hours of incubation, figure 3. The hypophysis anlage is closely applied to the brain wall, causing the nasal surface of the pouch to be sharply concave. The lateral lobes are more prominent than in the preceding stage. The lumen of the pouch extends well into each lateral lobe. One striking feature is the extensive degree of communication between the cavity of Seessel's pouch and the hypophyseal sac. The two open into each other almost to the summit of the ecto-entodermal fusion. This causes the opening of the hypophyseal sac into the oro-pharynx to be relatively larger than in previous stages. From an examination of embryos of this age alone the impression might be gained that the lateral lobes are being added to Rathke's pocket. A critical comparison of this and younger stages, however, indiates strongly that the lateral lobes of the chick do not arise independently of Rathke's pouch, but that they are formed from it. In this we support the observations of Rossi.

Chick embryo, 96 hours of incubation. The principal feature of interest in this stage is the beginning recession of Seessel's pouch and its separation from the hypophyseal sac. The lateral lobes are more sharply marked off from the superior part of the hypophysis, but otherwise this stage does not exhibit any striking differences from the preceding.

All figures represent wax-plate reconstructions of the epithelial hypophysis as viewed from in front and from the left side. *S*, Seessel's pouch, *R*, Rathke's pouch, *l.l.*, lateral lobes, *t.p.*, tuberal processes, *st.*, hypophyseal stalk.

Fig. 1. Hypophysis region from chick embryo of 21–2 pairs of primitive segments (end of second day of incubation). × 100.

Fig. 2. Hypophysis from chick embryo of 30 pairs of primitive segments (59 hours of incubation). × 100.

Fig. 3. Hypophysis from chick embryo of 72 hours of incubation. × 100.

Fig. 4. Hypophysis from chick embryo, 5 days (120 hours) of incubation. × 75.

Fig. 5. Hypophysis from chick embryo, 6 days (144 hours) of incubation. × 75.

Chick embryo, 5 days (120 hours) of incubation. By this time a definite hypophyseal stalk has been formed. It is hollow and affords a communication between the lumen of the hypophysis and the oral cavity. The lateral lobes have increased in size so that the transverse diameter of the gland, measured between the lateral extremities of the two lobes, is almost twice the transverse diameter of the superior part of Rathke's pocket. The lateral lobes are united by a prominent ridge around the inferior and nasal end of the hypophysis. This solid median protuberance doubtless corresponds to a vestigial 'Vorraum' or 'corpus lobuli bifurcati' of other vertebrates as described by Woerdeman. The lateral lobes are beginning to be solid, also. At this stage they sometimes contain lumina, which, however, no longer communicate clearly with the main hypophyseal cavity.

Seessel's pouch is represented by a solid bud of epithelial cells just dorsal to the hypophyseal stalk (S, fig. 4). Curiously enough Economo labels this bud the remains of *Rathke's* pocket.

Chick embryo, 6 days (144 hours) of incubation. The hypophyseal stalk is much elongated and has become solid. Near its connection with the oral epithelium may be seen the bud-like remains of Seessel's pouch. The superior, or distal, half of the hypophysisis is bent dorsally and forms an angle of about ninety degrees with the inferior or proximal half of the gland. The constriction near the middle of the gland is pronounced. Distinct 'tuberal processes' have formed from the lateral lobes. Instead of projecting so much laterally, they are now directed toward the brain wall. The tuberal processes are not located at the extreme nasal end of the gland but are seen to protrude from about the middle of the inferior half (fig. 5).

SUMMARY

The lateral lobes, from which the tuberal processes arise, may be distinguished in a chick embryo having 30 pairs of primitive segments. From a careful study of stages preceding and following the rupture of the oral membrane it is evident that the lateral lobes are not formed independently of Rathke's pocket

and later added to it, but are rather formed secondarily from the nasal wall of the early hypophyseal anlage.

The lateral lobes, in all forms studied, appear early in development. This would indicate that they and their derivative in higher vertebrates, the pars tuberalis, are of fundamental phylogenetic importance. Thus great interest is attached to the broad homologies drawn by Woerdeman ('14).

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THE IDENTIFICATION OF ENDOTHELIAL LEUCOCYTES IN HUMAN TISSUE

THIRD REPORT OF STUDIES ON THE MONONUCLEAR CELLS OF THE BLOOD

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TWO FIGURES

In an earlier report by the writer ('18) it was shown that the phagocytic mononuclear cells present in the peripheral blood arise by mitosis from the endothelium of the blood vessels. The method that was devised for this purpose consists of the intravenous injection of lampblack suspensions and is not, therefore, applicable to human tissues. The tissues of animals injected with carbon suspensions in which the endothelial leucocytes and cells are characterized by carbon particles ingested by phagocytosis are, however, well adapted for testing the action of various stains on these cells. It has been found that the staining method used by Graham ('16) colors these leucocytes in a characteristic way in both animal and human tissue. Since paraffin or celloidin sections cannot be used, Graham employed frozen sections for his stain but owing to their thickness, they are not suitable for accurate cell identification. The purpose of this paper is to record a new method of tissue imbedding for obtaining thin sections to which the stain is applicable.

The staining method of Graham depends on the action of solutions of alphanaphthol on parts of the cytoplasm of cells. It was shown by O. Witt ('82) that a blue dye (indophenol blue) is formed by the oxidation in dilute alkaline or acid solution of alphanaphthol and dimethyl-para-phenylenediamine. Winkler ('07) and others found that myeloblastic cells react in a characteristic way in tissues treated with these two substances and the

granules of the reacting cells were said to contain an oxidizing ferment (oxydase or peroxidase) that oxidized the two compounds and caused them to unite with the production of a blue color in the neutrophilic and eosinophil granules. Later Loele ('14) found that the treatment of myeloblastic cells with alphanaphthol solutions alone produced the same blue color in the granules.

The part played by the cell granules has usually been regarded as an oxidizing one (oxydase or peroxydase reaction). As applied by Loele the phenomenon consists of a purplish or bluish color in the cytoplasmic granules of the cell produced by treatment with old alphanaphthol solutions. If a dyestuff is produced by alphanaphthol alone, and this seems likely, an aromatic compound or compounds must be supplied by the leucocytic granules. If such is the case "indophenol reaction" is a better term than "oxydase reaction."

Leucocytic granule stain (Graham). Since hydrogen peroxide is added to the alphanaphthol solution to make it immediately active and the swollen granules are heavily and permanently stained by treating the preparations with an aniline dye, the method of Graham is better than the other indophenol staining methods devised. To apply this method, remove thin sections of formalin-fixed tissue attached to slides from the distilled water and stain in dilute (1-5) hematoxylin (Delafield) for five minutes; wash them in distilled water, place in a saturated solution of lithium carbonate for five minutes, wash in distilled water for two minutes, and stain for ten minutes in 10 cc. alphanaphthol solution to which 10 drops of 1 per cent pyronin (Grübler) have been added immediately before placing the preparations in it. Prevent evaporation by covering the dish. Wash the sections in distilled water, place in saturated aqueous solution of lithium carbonate for from five to ten minutes, wash in water and differentiate and dehydrate in 95 per cent alcohol for one-half minute. Complete the dehydration by immersing the slides in xylol and raising them above the surface of the liquid two or three times and blotting with smooth, soft filter paper. A flat oblong staining dish, the size and width of a slide, is used for the staining and differentiation of the slides. Mount in

colophonium-xylol or acid-free balsam. The time that the preparations remain in the saturated lithium carbonate after treatment with alphanaphthol is important because this removes the excess of pyronin. With some tissue better results are obtained by staining only five minutes in the alphanaphthol-pyronin solution and differentiating for a shorter time in the lithium carbonate after this stain. If the one per cent pyronin solution is to be kept for some time, sufficient formalin should be added to make it 10 per cent formalin.

The alphanaphthol solution is made by dissolving 1 gm. alphanaphthol (Merck Reagent) in 100 cc. 40 per cent ethyl alcohol (made from absolute alcohol) at a temperature of 50°C. and adding 0.2 cc. hydrogen peroxide. Commercial hydrogen peroxide containing approximately 3 per cent hydrogen peroxide, as determined by titration with decinormal potassium permanganate, should be used.

Soap method of imbedding. 200 grams transparent glycerine toilet soap are placed in a 500-cc. Erlenmeyer or Florence flask that contains 200 cc. distilled water. The soap must be so hard that it is brittle and cracks apart when cut with a knife, otherwise the soap solution will not be of the proper consistency. The flask is placed in the paraffin oven at 52°C. overnight in order to dissolve the soap. Remove it from the oven and place for three hours in an incubator at 37.5°C. The contents should be a syrupy liquid and should solidify when a small amount is poured into a paper boat and allowed to stand at room temperature for one-half hour. If solidification does not take place, 20 grams of soap should be added to the flask, and the contents again melted in the paraffin oven. After the correct consistency has been obtained the soap solution is placed in 100-cc. wide-mouth bottles with cork or glass stoppers, with about 50 cc. to a bottle.

To imbed the tissue small pieces are taken from 10 per cent formalin and dropped into the melted soap contained in one of the bottles which is placed at 37.5°C. two to three hours and occasionally shaken. The liquid soap in the bottles usually become solid after remaining at 37.5°C. for a day or more. To melt the solidified soap, the bottles are placed in the paraffin oven for

an hour, the soap cooled to 45°C., the tissue added, and the bottles replaced in the incubator at 37.5°C. The soap solution with the tissue in it is emptied into a box of suitable size made from paper as in paraffin imbedding and the tissue arranged on the bottom of the box with forceps. The box should be made from paraffined paper or the paper may be coated by pouring melted paraffin into it. At the end of about one hour the paper is removed, the solid soap trimmed with a knife to the desired size about the tissue and the blocks attached to a heated metal disk just as paraffin blocks are attached. The blocks after about one hour are dropped into a saturated solution of sodium chloride in a pint Mason jar, and the jar placed in the incubator at 37.5°C. overnight.

With forceps remove the block from the saturated salt solution, attach to rotary microtome and cut away the block until the tissue is reached. Carefully trim the block and allow to dry for from three to six hours until a ribbon 6 to 8 microns thick cuts perfectly. The disks may be detached from the microtome and after the proper drying again attached, so that the ribbon comes from the very surface of the block. The ribbon is placed in distilled water in a flat dish more than 6 inches in diameter, and sections floated on slides on which there is a thin coating of fixative made by adding 4 cc. of a very thick syrupy celloidin to 16 cc. oil of cloves. The preparations after being pressed out and carefully blotted with filter-paper are placed in the paraffin oven for fifteen minutes, when they are removed, washed in 95 per cent alcohol for thirty seconds, and placed in distilled water where they remain less than five minutes. Ionization in the large volume of water in which the soap sections are first placed develops only a slight alkalinity, and in the thick soap solution ionization is practically absent. The saturated salt solution hardens the blocks since it prevents hydrolysis by mass action. If the tissue is quite fragile the ribbon may be placed in saturated salt solution instead of distilled water.

Reaction of sections obtained by the soap method of imbedding to the stain. The nuclei are blue and the granules of myeloblastic cells, endothelial cells and endothelial leucocytes are red. Graham

noted the red granules in both endothelial cells and endothelial leucocytes and explained their presence there as the result of ingestion by phagocytosis of myeloblastic cells or the cytoplasmic granules of these cells. He does not make it clear whether the granules were found in all endothelial cells and leucocytes.

In the thin soap sections it is evident that many cells of endothelial origin contain the granules and that they are not present here as the result of an accidental phagocytic phenomenon is shown by the small size of the granules and their even distribution in the cell cytoplasm. The granules differ from the myeloblastic granules seen in neutrophiles and eosinophiles in being fewer in number, smaller in size and more discretely distributed. In tissues in which only mature (polymorphonuclear) myeloblastic cells are present a casual glance is sufficient to distinguish the endothelial leucocytes containing the red granules since they are mononuclear. In tissue containing myelocytes, the heavier staining and greater number of granules of the neutrophilic and eosinophilic myelocytes separate them from the endothelial leucocytes. The differentiation between myelocytes and endothelial leucocytes is well shown in sections of bone-marrow (fig. 2).

The characteristic action of the stain is best seen in sections of the liver or other organ of an animal that has received intravenous injections of a lampblack suspension according to a method devised by the writer ('18). Endothelial cells containing carbon and definitely lining the sinusoids have red granules scattered in their cytoplasm (fig. 1). Likewise carbon-containing endothelial leucocytes in the vessels show the discrete red granules. The neutrophilic and eosinophilic leucocytes are more conspicuous than the cells of endothelial origin owing to the greater number and larger size of their granules. There are a certain number of neutrophiles, eosinphiles, and endothelial leucocytes that do not take the stain; and it is only in the endothelial cells with a distinct amount of visible cytoplasm that the granules may be distinguished. The failure of some cells to stain appears to be due to an error in technic but all attempts to correct this have failed.

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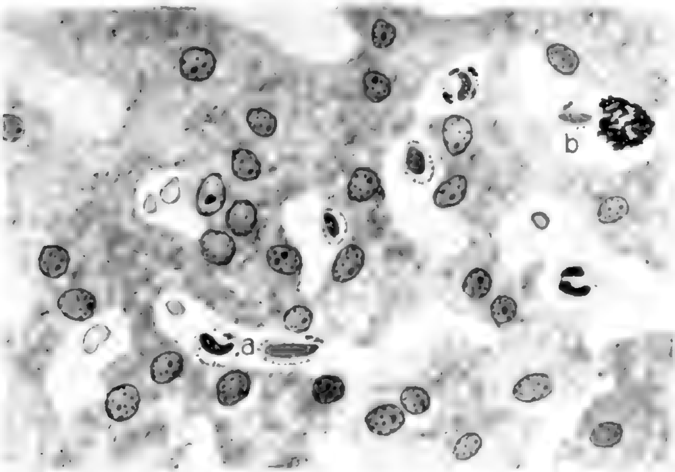
PLATE 1

EXPLANATION OF FIGURES

Both figures drawn with camera lucida and oil-immersion lens from indophenol stains of 7-micron soap sections.

1 Liver of a dog that has received intravenous injections of lampblack suspensions. *a*, two endothelial leucocytes containing small particles of carbon; *b*, an endothelial leucocyte near a mass of carbon in a sinusoid.

2 Bone-marrow (human). *a*, endothelial leucocyte or cell, *b*, myelocyte; *c*, polymorphonuclear neutrophile. All leucocytic granules are a bright red; those in the cells of endothelial origin are smaller and more discrete than those in the myeloblastic cells.



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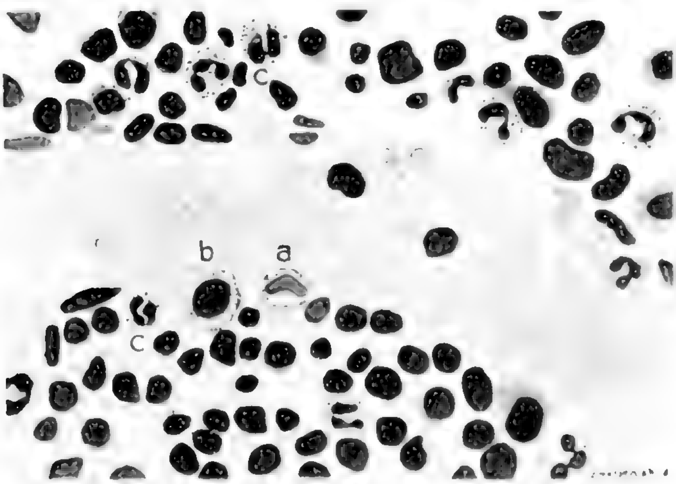

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, OCTOBER 8

THE SCAPULA OF TRAGULUS

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NINE FIGURES

During the summer of 1917 the author was examining the collection of mammalian scapulae in the U. S. National Museum, Washington, D. C., seeking data in regard to an entirely different problem than the subject of this present paper. While handling this material his attention was attracted to a scapula, whose suprascapular region was quite unlike anything else in the whole mammalian series under review.

This scapula was of an adult mounted specimen of one of the species of Tragulus; also known as Pigmy Deer, Deerlet, and Chevrotain. There is very little in the literature concerning these small deer-like animals, which have also some points in common with both pigs and marsupials. Their systemic position is doubtful, but Beddard ('02), whose brief account is the most extensive one known to me, puts them with the Ruminants since they possess Artiodactyle feet. In many respects, dentition, feet, stomach, and placenta, they are intermediate between the pigs and the Ruminants.

Milne-Edwards ('64) has a few notes on the structure of these animals.

Parker ('68) in his Monograph on the Shoulder-Girdle and Sternum, does not figure the scapula of Tragulus at all, but gives the following brief, and with one exception, correct account of it:

In an adult Tragulus javanicus the whole shape is broader, the neck short, and very narrow; the deep spine forms a right angle with the suprascapular border of the bone: this border is nearly straight; the praescapular region has an arcuate outline, and its fossa is only one-fifth the width of the widest (upper) part of the infra-spinous space.

The suprascapula ossifies very late; the spine grows downwards into a straight, sharp acromion; the coracoid process is well shown, but is short, flat, emarginate, and incurved.

The exception taken to Parker's account is regarding the ossification of the suprascapula. The authorities of the U. S. National Museum placed at my disposal several specimens of foetal and adult Tragulidae, four of which are seen in figures 1–4.

Figures 1 and 2 are drawings of foetal scapulae of Tragulus, one from Borneo, and the other from S. W. Borneo. They were from foetuses about $4\frac{1}{2}$ inches in length, and there is no essential difference between the two specimens. The suprascapula is relatively large and as yet no center of ossification is present. The entire shape is essentially adult, except that the cartilaginous region around the glenoid fossa extends some little distance up the neck of the scapula; also the neck of the scapula of figure 1 is much thicker than in the adult.

Figures 3 and 4 are of adult scapulae. The first one is from Borneo, the second from Java. Here appears the peculiarity in the suprascapula, of which this paper is a brief record. In the center of the cartilaginous supra-scapula and occupying roughly one-third its area is a patch of bone. The bone is entirely surrounded by cartilage of the suprascapula, and as the animals were obviously fully matured, it is not likely that this center of bone formation ever unites with the scapular blade.

The fact has been mentioned that in several respects Tragulus is intermediate or transitional between the Suidae and the higher Ungulates. It would seem that in the scapula also we have another character pointing to this intermediate position. In figures 5–9 are shown a series of vertebral borders of scapulae, beginning with the pig in which there is never any ossification in the suprascapula, not even in an aged boar, as I have recently had the opportunity of ascertaining. In this series of sketches (fig. 5–9) Tragulus seems to fit in between the Suidae and the Red Deer, in which the suprascapula is poorly ossified over its entire extent (Flower); this in turn leads to the Carnivora, with the merest tip of cartilage remaining; and so on up to the Primates, where the vertebral border has lost the last vestige of cartilage in the mature adult.

Thus in an ascending phylogenetic series of animals the scapulae arrange themselves with regard to a diminishing suprascapula in the precise manner in which this reduction takes place in the ontogeny of the scapula in any one of the higher mammals.

I desire to express my deep appreciation of the courtesy accorded me by Mr. Gerritt S. Miller, Curator of the Division of Mammals, U. S. National Museum, in permitting me to examine and dissect this rare and valuable material.

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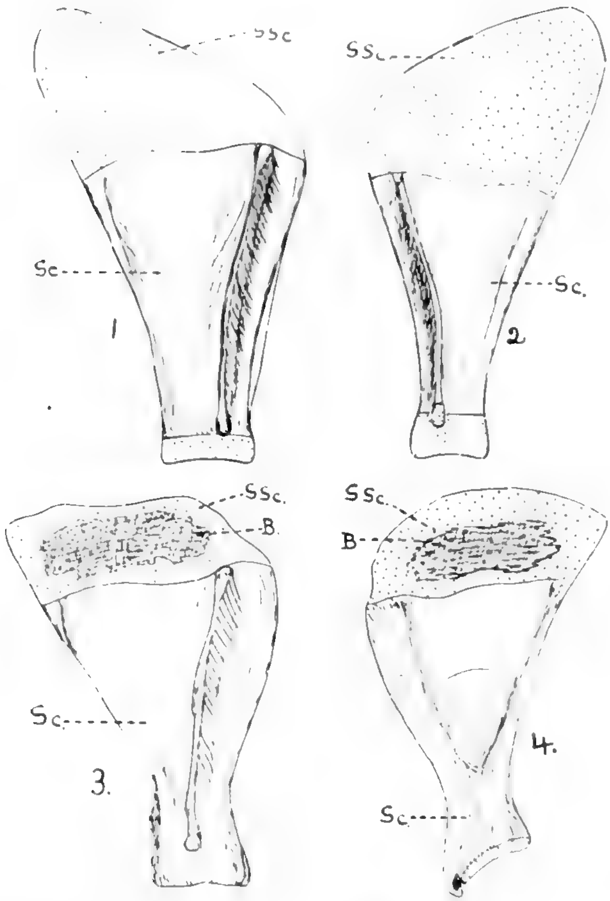


Fig. 1 Scapula of foetus of Tragulus from Borneo, medial side. × 8. U. S. Nat. Mus. specimen No. 173.

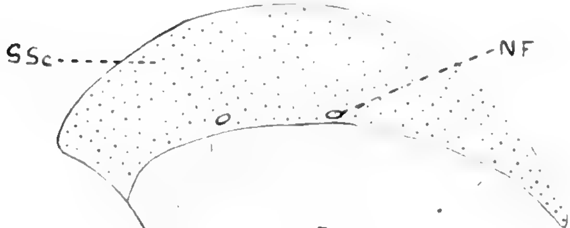
Fig. 2 Scapula of foetus of Tragulus from S. W. Borneo, lateral side. × 8. U. S. Nat. Mus. specimen No. 153966.

Fig. 3 Scapula of adult Tragulus from Borneo, showing bone formation in centre of cartilaginous suprascapula. × 1. U. S. Nat. Mus. specimen No. 197672.

Fig. 4 Scapula of adult Tragulus from Java. × 1. U. S. Nat. Mus. specimen No. 156289.

ABBREVIATIONS

B, Bone	*SSc.*, Suprascapula
N.F., Nerve foramina	*Sc.*, Scapula



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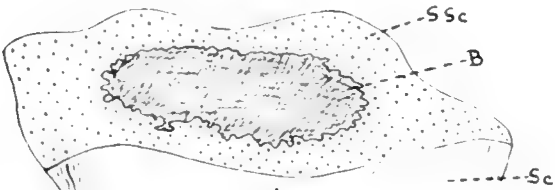

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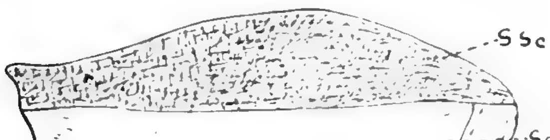

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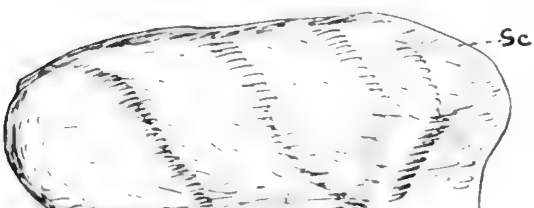

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Fig. 5 Vertebral border of adult pig. Suprascapula never ossifies. Two branches of spinal nerves pass through foramina in the cartilage.

Fig. 6 Enlarged view of vertebral border of scapula in figure 3.

Fig. 7 Suprascapula of Red Deer. Modified after Flower.

Fig. 8 Vertebral scapular border of adult male cat.

Fig. 9 Vertebral scapular border of man.

Resumido por el autor, Waro Nakahara.

Algunas observaciones sobre el crecimiento de los ovocitos de Perla inmarginata Say, con especial mención del origen y función de las estructuras nucleolares.

El núcleo del huevo ovárico de Perla inmarginata contiene dos clases de estructuras nucleolares, a saber: Un nucleolo único, de gran tamaño (nucleolo principal) y otro múltiple mas pequeño (accesorio). Las observaciones efectuadas por el autor indican que el primero existe en el núcleo desde los primeros estados del crecimiento, pudiendo emigrar algunas veces, fuera de él demostrando esto su origen endogénico. Los nucleolos accesorios se derivan, con toda probabilidad, de los núcleos vitelinos que se encuentran primero en el área citoplásmica y más tarde penetran dentro del núcleo. Estos hechos demuestran que las teorias endogénica y exogénica de las estructuras nucleolares, no son necesariamente contradictorias. Si consideramos las estructuras nucleolares como representación de substancias que pasan a través del núcleo durante el metabolismo, los nucleolos endogénicos deben considerarse como pertenecientes al lado catabólico, los exogénicos al lado anabólico del proceso.

Translation by Dr. José Nonidez,
Columbia University.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, NOVEMBER 18

SOME OBSERVATIONS ON THE GROWING OOCYTES OF THE STONEFLY, PERLA IMMARGINATA SAY, WITH SPECIAL REGARD TO THE ORIGIN AND FUNCTION OF THE NUCLEOLAR STRUCTURES[1]

WARO NAKAHARA

Biological Laboratories, Cornell University, Ithaca, N. Y.

NINE FIGURES

CONTENTS

INTRODUCTION

There are two conflicting views regarding the origin of the nucleolus. According to Korschelt ('89) and Montgomery ('98), the nucleolus represents a sort of nutritive substance derived from the cytoplasm, and therefore it is extranuclear in origin: while, on the contrary, a number of recent authors, including Obst ('99), Walker and Tozer ('09), et al., maintain that it is of intranuclear origin. The general contention today that the nucleolar substance is a passive product of the nuclear activity, and hence intranuclear in origin, seems premature, in view of the fact that data cited as supporting the exogenic theory of the nucleolus have not been adequately studied.

In the course of my study of the ovarian eggs of the common stonefly, Perla immarginata Say, I found some facts which are

[1] In the preparation of this paper, the author is under deep obligation to Dr. Wm. A. Riley, now of the University of Minnesota, for the critical examination of the manuscripts.

directly related to the question at issue, and are of a little value, if my interpretation is correct, in showing the possibility of bringing the two conflicting views in harmony.

The following note summarizes the results so far obtained in the study, which has been carried on in the biological laboratories of Cornell University, until it was discontinued through my leaving the institution. Although there are many details remaining to be worked out, points brought out in the following lines seem clear.

TECHNIQUE

Although several other fixing fluids were employed, the one that proved to be most satisfactory and hence most used is Flemming's chromo-aceto-osmic. Ovaries, dissected out in the normal salt solution, were fixed in this fluid for twenty-four hours.

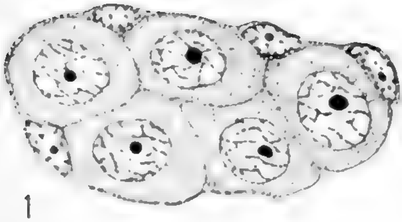
For staining, the best result was obtained by a modification of Flemming's "triple" method, which is as follows:

1. Stain in a mixture of equal parts of saturated aqueous and saturated alcoholic solutions of safranin for five minutes.
2. Rinse in water.
3. Stain in 1 per cent gentian-violet, about ten seconds.
4. Rinse in water.
5. Stain in 2 per cent solution of orange G for two or three minutes.
6. Rapidly dehydrate with absolute alcohol; differentiate with clove oil under the control of microscope; clear with xylene; mount in balsam.

Iron hematoxylin and borax carmine counterstained with Lichtgrün were also used for a few slides.

OBSERVATIONS

Figure 1 represents a group of early oocytes and some follicular cells. In the nucleus of the oocyte, the chromatin granules, which stain deeply with gentian-violet, are seen arranged more or less linearly. The nucleolus is very prominent, always being situated near the center of the nucleus. Before the growth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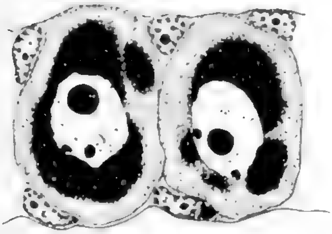
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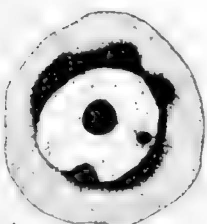
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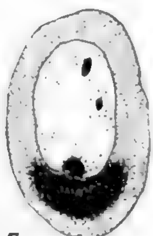
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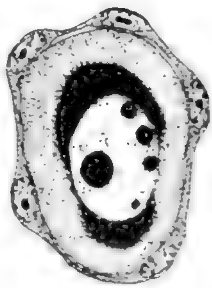
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Fig 1. A portion of 'egg tube,' showing early oocytes with prominent nucleolus. × 670.

Fig. 2. A portion of 'egg tube,' showing early oocytes and a growing oocyte Two early oocytes are seen in the process of degeneration. × 670

Figs. 3 to 6. Early ovarian eggs. In figures 4 and 5 follicular cells are not shown. The yolk-nuclei are represented by black masses around the nucleus. The single nucleolus is seen near the nucleus, except in figure 5, which does not show this structure. In figure 4 a peripheral nucleolus is seen half way through the nuclear membrane. × 670

of the oocytes commences, some of them always seem to be committed to degeneration. In figure 2 two cells are shown to be in this process. The nuclear structure of the normal oocyte at this period is very much the same as before.

In its very early state (figs. 2 to 6) the ovarian egg is more or less irregularly oval. Follicular cells surrounding the eggs are few in number. The nucleus of the egg cell is usually oval or spherical, containing chromatin granules, which stain less intensely than in the oocyte. Nucleoli are of two sorts, a large principal nucleolus and a number of smaller nucleoli. The larger nucleolus, which seems doubtlessly derived from the original oocytic nucleolus, is almost always single and is situated near the center of the nucleus. Its substance is usually homogeneous, with clearly defined outline, taking acid color (orange G) when treated with Flemming's triple stain, and seldom containing a vacuole.

The smaller nucleoli vary in number; a section usually showing two or three, but sometimes more—up to six or seven. They vary also in size, although none of them were found to be even half as large as the larger nucleolus. In shape, they are in most cases spherical, or more rarely much elongated. They are very characteristic in being situated just beneath the nuclear membrane, or at least not far from the latter. The substance of the smaller nucleoli distinguishes itself from that of the larger nucleolus in choosing safranin out of Flemming's triple combination.

The most characteristic structure in the ovum of this stage is the irregular masses of dense material in the cytoplasm, always closely apposed to the nucleus. Whether the masses in question, which are apparently identical with a certain kind of yolk-nuclei, lie within or without the nucleus, is in many cases quite difficult to determine. For instance, in two cells represented in figure 3, it has been impossible for me to detect the nuclear membrane between the areas of the nucleus and yolk-nucleus. It is still more interesting that the substance of the yolk-nuclei agree with that of the smaller nucleoli in staining reaction. These facts, taken together with the peripheral posi-

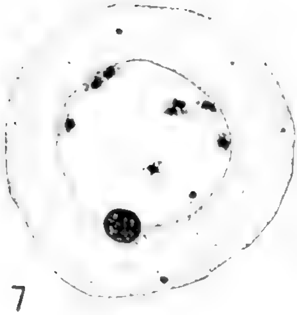
tion of the nucleoli, are suggestive enough of some very close relation between these two kinds of structures.

Ova of the next stage in the growth are represented in figures 7 and 8. Here the increase in size is most marked in the nucleus, although the follicular epithelium is also attaining much development. The larger nucleolus is somewhat larger than in the earlier stage, but in other respects, it does not show any appreciable difference according to the stages. It may, however, sometimes migrate out of the nucleus in the well-known manner for the phenomenon (fig. 7). The smaller nucleoli are seen to be much increased in number, usually about twice as many as in the last stage, and not infrequently more than fifteen, being present in a single section. There is no 'yolk-nucleus' situated close to the nucleus. Sometimes, as represented in figure 8, less deeply stained and rather indistinct masses may be seen scattered near the periphery of the cell, but this is not a constant feature of the ovum at this stage. The nature of these masses is unknown, but they may be, in some way, connected with the yolk-nuclei. A few yolk-granules are seen to appear in the cytoplasm (fig. 7).

This stage of growth is followed by another, at which the volume is increased in the cell-body, rather than in the nucleus. At this stage, the follicular epithelium is attaining its full growth (fig. 9). The nucleus remains in nearly the same size as in the preceding stage and the larger nucleolus shows no special change. Most of the small nucleoli are now moved from the periphery toward the center of the nucleus, and they are somewhat less in number than in the last stage. The cell-body grows immensely. Indeed, the growth of the cell at this stage may be almost entirely attributed to that of the cell-body. The cytoplasm is rather homogeneous, and no structure resembling the yolk-nucleus is found. The yolk-granules are present in a fairly large number at this stage.

GENERAL CONSIDERATION

From the facts described in the last section, it is evident that the growth of the ovarian egg of Perla is effected first by a rather marked growth of the nucleus, and then by an immense increase of cytoplasm.



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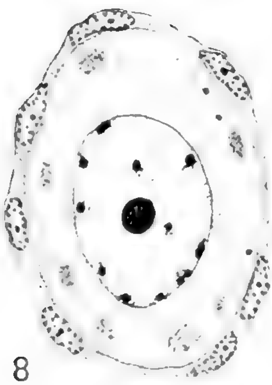

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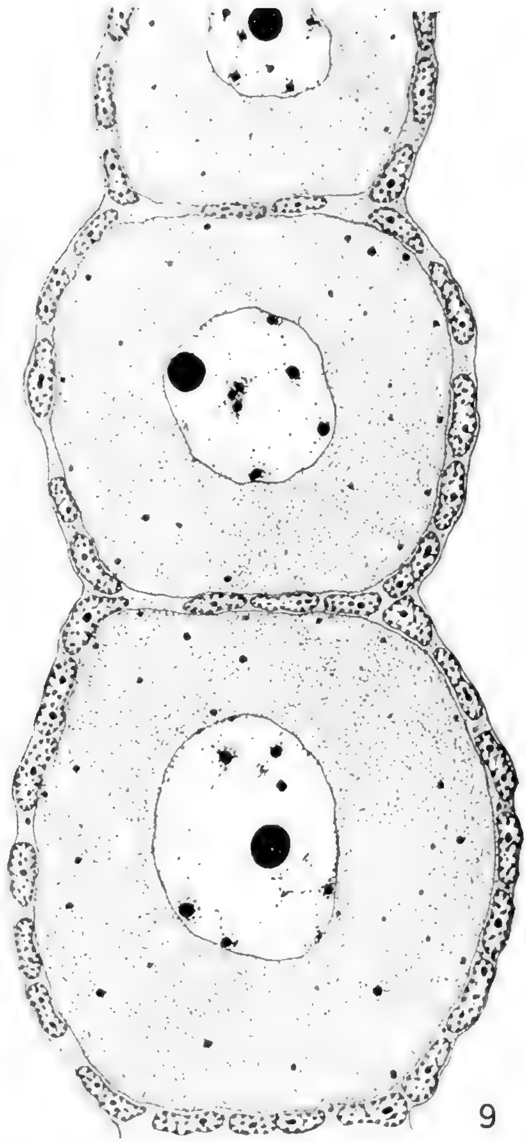

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Fig. 7 An ovarian egg in the stage of nuclear growth; follicular cells are not drawn. Note the increase of the smaller nucleoli. The larger nucleolus is here seen passing out into the cytoplasm. × 670.

Fig. 8. The same stage. Indistinct dark masses are seen scattered in the cytoplasm. × 670.

Fig. 9. Ovarian eggs in the next stage. The cell-body is greatly increased in volume. Note the less numerous smaller nucleoli. Deposition of yolk-granules in the cytoplasm is also illustrated. × 670.

The appearance of the 'yolk-nuclei' always precedes the nuclear growth. During the growing period of the nucleus, the yolk-nuclei more or less diminish and finally disappear, and the growth of the cytoplasm is rather insignificant.

It has been observed that the yolk-nucleus and the peripheral nucleoli show very similar staining reactions, and that in some cases the former is so closely apposed to the nucleus as to make it impossible to draw a line between the nuclear area and the area of the yolk-nucleus. In fact, many cases were found where the only possible interpretation was that the peripheral nucleolus and the yolk-nucleus are the same substance, and it may pass through the boundary of the nucleus when the latter is not provided with nuclear membrane. As far as these facts are concerned, it might be considered either, 1) that the nucleoli migrate into the cell-body and give rise to the yolk-nuclei, or, 2) that the latter are taken up by the nucleus and constitute the former there. However, since the yolk-nuclei appear preparatory to the growth of the nucleus, the first theory does not seem to be acceptable; therefore, the substance of the yolk-nuclei is to be considered as, at least in part, a provision for the growth of the nucleus, to be taken up by the latter to constitute the peripheral nucleoli.

In this connection, a few words might be said regarding interpretation of the 'yolk-nuclei.' The data accumulated by Hubbard ('94), Calkins ('95), Henneguy ('96), Foot ('96), Němec ('97), Van Bambeke ('98), Munson ('98) and Crampton ('99) from various animals indicate, in a general way, that the yolk-nucleus and a certain nuclear substance are very closely allied to each other, if not exactly identical, and that later the yolk-nucleus breaks up into smaller and smaller granules, which scatter through the cell-body. Wilson ('00), in his excellent review, expresses his opinion that the yolk-nucleus may be a product of the nuclear activity, being directly or indirectly derived from the nucleus, and it many contribute to formed elements of the cytoplasm.

In his extensive comparative study on the subject, Munson ('12) speaks of the type of yolk-nuclei under discussion as

'metaplasm.' According to him, the metaplasm is a product of ferment action of karyolymph, which comes out of the nucleus, upon unassimilated, ingested food in the cytoplasm, and it is gradually absorbed as food by the sphere.

To the case of Perla egg, no one of these interpretations is applicable, because here, in all probability, the yolk-nuclei are, at least in part, provisions for nuclear growth. It may be that the yolk-nuclei in this case are derived from the degenerating cells, which are rather abundant at the time when the growth of the ovum starts, and they may be entirely different in nature from the yolk-nuclei of other forms studied. However, the whole subject obviously needs a careful re-examination. Regarding the larger nucleolus, nothing very definite can be said as to its origin. Indications as a whole, however, seem to favor the view of its intranuclear origin. It is very probable that the nucleolus was formed in the nucleus after the last oogonial division, and handed down through the stages of growth of the ovarian egg.

REVIEW AND DISCUSSIONS

Korschelt ('89), working on the ova of Epeira, Dolomedes, Phalangium, Spinther, and Ciona, came to the conclusion that the nucleolar substance is in close connection with the nutritive process of the cell, and probably it is derived from the cytoplasm.

This theory is strongly maintained by Montgomery ('98), on the basis of his observations:

> In all the cases observed by me, the nucleus appears to assimilate a substance or substances from the cytoplasm, and after this substance has entered the nucleus it apparently undergoes there a chemical change, and becomes deposited on the inner surface of the nuclear membrane in the form of masses of varying dimensions, which may be either globular or irregular in shape, according as they are fluid or viscid in consistency. In the case of the ova of the nemerteans, the substance taken up into the nucleus, and which there becomes deposited in the form of nucleoli, is sometimes exactly similar to the substance of the yolk-balls, which lie in the cytoplasm; in other cases, it is probably similar to those metabolically changed portions of or inclusions of the cytoplasm, out of which the yolk-balls are later differentiated. In Lineus, indeed, the yolk-balls may often be found halfway through the nuclear membrane, and their appearance is

exactly similar to that of the nucleoli. In the mesenchym cells of Ceregratulus, the substance of the nucleoli appears to be identical with that of the numerous nutritive granules which are dispersed in the cytoplasm; the latter globules arise in the cytoplasm before the nucleolus appears in the nucleus, and as soon as they become numerous in the neighborhood of the nucleus, peripheral nucleoli begin to appear in the latter. In the subcutical gland cells of Piscicola, the nucleolus, at the time of its most rapid growth, is apposed to the nuclear membrane; but when this period of volume-increase has ceased, it is never found in this position. Furthermore, the paranucleoli of Rodalia appear first in contact with the nuclear membrane.

The greater part of the above statements are in accordance with my observation on Perla egg, and in my mind there is little doubt as to the appropriateness of Montgomery's interpretation. Regarding the case of subcutical gland cells of Piscicola only, I am rather inclined to disagree with him, because of the fact that the nucleoli migrate into cytoplasm in great abundance at the time of the formation of secretion granules—a fact which apparently speaks against the theory of extranuclear origin of the nucleoli. Montgomery also cites Schwalbe's ('76) observations that in some vertebrate embyros, the nucleoli first arise as thickenings of the inner surface of the nuclear membrane. However, any literature published at as early a date as 1876 on 'nucleolus' can hardly be expected to be of much value on account of the poor technique employed and indefinite use of the term 'nucleolus,' so such early observations as these are perhaps to be intentionally excluded from our consideration.

Obst ('99) considered that the nucleoli are intranuclear in origin and, more especially, they are derived from the chromatin indirectly by some chemical change. He observed in Unio, Epeira, and other forms that in resting nuclei the basophile chromatin granules become acidophile, and then they fuse together to form nucleoli.

Page and Walker ('08), in the nerve cells of several mammals, Walker and Embleton ('08), in the cells of Hydra, described the multiplication of the nucleoli by regular budding. The nucleoli then pass out of the nucleus and eventually become absorbed. Walker and Tozer ('09) described similar observations in the

vegetative cells from Spongilla, Planaria, Clepsine, the rabbit, and the bean plant, and stated that "the regular multiplication of nucleoli within the nucleus, and their subsequent history, seems to exclude the possibility of the extra-nuclear origin suggested by Montgomery," at least in the case of the cells they considered. A similar conclusion may be reached also in the case of silk-gland cells of insects, since the numerous nucleoli in functional cells are derived from a single nucleolus of ordinary type by budding (Vorhies, '08), and they then pass out into the cell-body to form secretion granules (Maziarski, '11, and Nakahara, '17).

Medes ('04) and Dederer ('07), in the male germ cells of Scutigera and Philosamia, respectively, observed that the true nucleolus first appears in a mass of chromatin. McGill ('06), studying the ova of the dragonfly, expresses her opinion that the double nucleoli in that form are formed through the condensation of the basichromatin round the oxyphile nucleolus. Wilson ('05), Boving ('07), Randolph ('08), Stevens ('08), and Payne ('09), in the male germ cells of various insects, observed that the sexchromosomes are usually closely associated with the nucleolus, when they first appear. The idea of a close genetic connection between the nucleolus and at least of a certain chromosome thus developed has been finally put in a somewhat definite form by Goldsmith ('16), who concludes, from his study on the spermatocytes of Pselliodes, that "the true nucleosus is formed by the accumulation of linin about the sexchromosomes."

It is very unfortunate that many of the recent authors are rather too skeptical toward Montgomery's theory. Goldsmith ('16) went even so far as to say that the peripheral position of the nucleoli in the nucleus was the only evidence of the theory set forth by Montgomery, and that since many nucleoli do not come in contact with the nuclear membrane throughout their entire history, the evidence does not hold. The more recent author seems to have forgotten that there are some other facts, correlated with the peripheral position of the nucleoli, pointed

out by the other author, in making his suggestion. If anyone goes over Montgomery's work very carefully he would find it impossible to escape from the conclusion that the 'extranuclear theory' is undoubtedly acceptable, at least in certain cases.

I have stated that in the ovarian egg of Perla the nucleoli are of two types, namely, a large single nucleolus and a number of smaller ones. The former increases in size with the general growth of the egg, and sometimes passes out into the cell-body, thus indicating its possible intranuclear origin. The nucleoli of the second type are absent in the early oocyte, but they are newly introduced in the nucleus at the time when the 'yolk-nuclei' make their appearance around the latter, and are in all probability directly derived from such extranuclear substance.

These facts suggest that the nuclear structures designated as nucleoli can be divided into two classes, according to whether it is derived directly from outside of the nucleus, hence probably associated with anabolism, or is secondarily produced within the nucleus from substances which have already been there, hence in probable connection with katabolism. A hypothesis might be put forth that *the nucleoli represent substances that are going through the nucleus in metabolism.* Nutritive substances taken up into the nucleus may or may not be in the form of distinct bodies, and when they are visible as such in sections, they constitute the nucleoli of extranuclear origin. For the nucleoli of intranuclear origin, the current interpretation that they represent a passive product of the nuclear, or more especially chromatin activity, may be a proper explanation. Viewed from this standpoint, the two apparently conflicting views are nothing but partial expressions of a whole truth, and recent authors' critical remarks upon Montgomery's conclusion are just as unrational as the latter author's premature generalization.

Cases where the extranuclear origin of the nucleoli is demonstrated are not abundant. Aside of those early ovarian eggs of certain animals, Conklin's ('97) figure of a cell from the dorsal wall of isopod intestine, showing nutritive substances projecting

by many processes into the nucleus, some of which apparently are being set free into the nucleus to form nucleoli, is the only published observation I know of this phenomenon.

SUMMARY AND CONCLUSIONS

1. In the ovarian egg of Perla immarginata, two types of nucleoli are present, namely, a large single nuceolus and a number of smaller peripheral nucleoli.

2. The larger nucleolus increases in its dimension with the general growth of the ovum, and it may sometimes pass out into the cell-body hence, possibly of intranuclear origin.

3. The smaller nucleoli are in all probability formed directly of the portions of the yolk-nucleus migrated into the nucleus—hence, extranuclear in origin.

4. Different views concerning the nucleoli, and especially the question of their origin, can be brought into harmony under the hyphothesis that the nucleoli represent substances going through the nucleus in metabolism.

5. According to this hypothesis, nucleoli may be formed directly of a material taken up by the nucleus or may be produced from some substances within the nucleus in the course of metabolic processes.

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Resumido por el autor, H. R. Hunt.

Variabilidad de las arterias carótidas comunes del gato doméstico.

El autor ha examinado ventiocho gatos, para determinar los limites y frecuencia de las variaciones del origen de las arterias carótidas comunes. En seis casos (22 por ciento) la arteria innominada se dividia en dos ramas, la subclavia derecha y un vaso que se bifurca en las dos arterias carótidas comunes. En nueve ejemplares (32%) la subclavia derecha y las dos carótidas comunes tenian un mismo origen en el extremo distal de la arteria innominada. En doce gatos (43%) la carótida común izquierda se originaba en varios puntos posteriores al orígen de la carótida común derecha. Un ejemplar macho presentaba una condición anómala bien patente, puesto que la carótida común izquierda partia directamente de la aorta, de un modo muy semejante a la disposición existente en el hombre. El autor propone una explicación de la variabilidad del orígen de las carótidas comunes.

Translation by Dr. José Nonidez
Columbia University

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, NOVEMBER 18

VARIABILITY IN THE COMMON CAROTID ARTERIES OF THE DOMESTIC CAT

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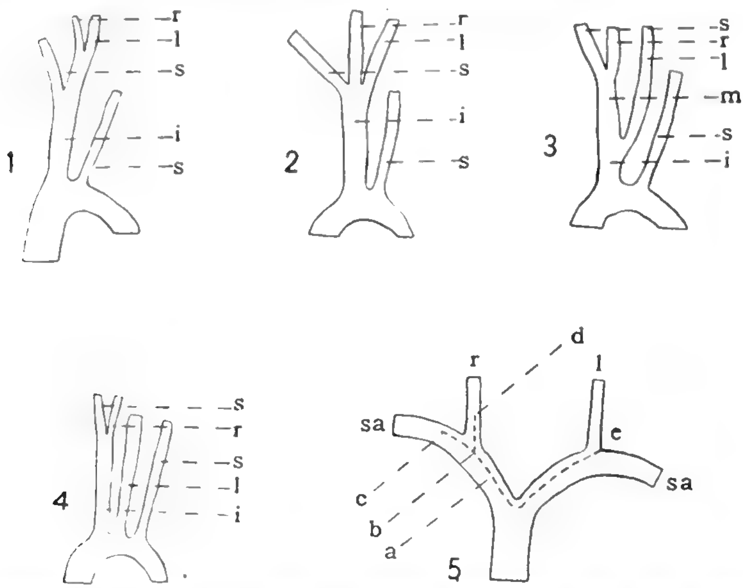
FIVE FIGURES

Variations in the origin of the common carotid arteries of the domestic cat have long been known. The conditions shown in figures 1, 2, and 3 are the usual variations and have been figured and described in the text-books on the anatomy of the cat. (Mivart, '81; Reighard and Jennings; Davison, '03.)

Twenty-eight cats have been examined to determine more precisely the limits and frequency of the variations in the origin of the common carotids. These individuals were of both sexes and being selected at random showed a considerable degree of variation in age and size. In six cases (22 per cent of the total number) the innominate artery split into two branches, the right subclavian and a vessel which divided into the two common carotids (fig. 1). Nine individuals (32 per cent) showed the conditions illustrated in figure 2, where the right subclavian and the two carotids have a common origin at the distal end of the innominate artery. In twelve cats (43 per cent) the left common carotid arose from the innominate at various points posterior to the origin of the right common carotid (fig. 3); in several animals the left carotid came off as far forward as *m*.

In one male animal a distinctly anomalous condition was found which does not appear to have been previously reported. The left common carotid of this animal was attached directly to the aorta (fig. 4), thus closely resembling the condition found in man. The above facts seem to show that figures 1 and 4 represent the extreme limits of variability in the origin of the common carotid arteries.

The cause of these variations is probably some variable developmental factor. Possibly the history of the left common carotid in the cat closely resembles that in the pig, where the left carotid is at first connected with the left systemic arch, but later shifts to the right arch (Lehmann, '06). Such a migration of the left carotid of the cat might, conceivably, be accomplished in the embryo by a splitting, of variable extent, of the left systemic arch, beginning at point *e*, figure 5. The condition shown in figure 3 would obtain if the split extended to point *a* in figure 5. Figure 2 would be produced by a further split to point *b*, while a split either to *c* or *d* would result in the conditions shown in figure 1. This hypothesis requires testing, of course, by studies on the embryology of the cat.



Figures 1, 2, 3, and 4 are sketches made from actual dissections. Figure 5 is a diagram to illustrate the hypothetical cause of the variation in the carotids. *i*, innominate artery; *l*, left common carotid artery; *r*, right common carotid artery; *s*, subclavian artery; *sa*, systemic arch.

Whatever the cause of these variations may be, the above observations indicate the frequency and limits of variability.

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Resumido por el autor, H. R. Hunt.

Ausencia de un riñón en un gato doméstico.

El gato macho estudiado por el autor carecía hasta del más ligero vestigio de riñón en el lado derecho del cuerpo, si bien existian en dicho lado la glándula adrenal, el testículo correspondiente y un uréter muy corto. El autor no ha podido hallar ni arteria ni vena renales. El riñón izquierdo estaba muy hipertrofiado.

Translation by Dr. José Nonidez,
Columbia University.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, NOVEMBER 18

ABSENCE OF ONE KIDNEY IN THE DOMESTIC CAT

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TWO FIGURES

The absence of one kidney, its ureter, and other parts of the urogenital system has been found in many human individuals by Schäffer, Ballowitz and others. v. d. Broek ('07) has reported a human male subject in which the whole right half of the urogenital system was absent. Lyon ('17) has described similar conditions in a female human individual, which, however, possessed a ureter on the side of the body lacking the other urogenital organs.

Recently the writer found and dissected a full grown male cat (cat 1) in which not even the slightest vestige of the right kidney was visible to the naked eye in the position normal to this kidney (fig. 1). The testes and the adrenal glands were present. The right ureter, *b*, was attached to the urinary bladder in the normal fashion; it ran cephalad for about 1.5 cm., and ended abruptly, showing nothing at its anterior end which suggested an undeveloped or degenerate kidney. It is evident that the agencies which prevented the normal development of the right ureter and kidney began to operate after the ureteric evagination of the Wolffian duct had been formed in the embryo.

Neither the right renal artery nor the corresponding renal vein could be identified. Vein *f* (fig. 1) drained the region supplied by the adrenolumbar artery, and is therefore the adrenolumbar vein, though it does not cross the adrenal gland as normally.

As far as could be determined by a gross dissection, the internal structure of the left kidney was normal.

The size of the kidney of cat 1 and of the kidneys of several normal male cats (cats 2, 3, 4 and 5) was determined by immersing the kidney in a graduate containing water, and then reading

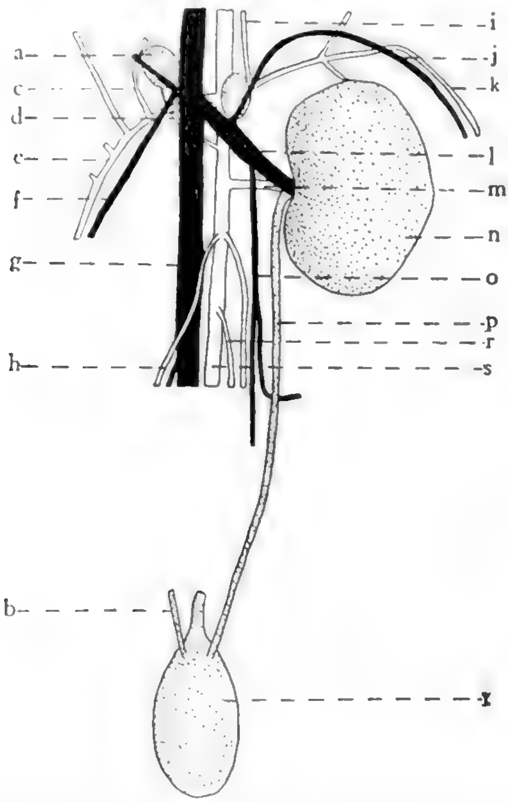

Fig. 1 *a*, adrenal gland. *b*, right ureter. *c*, right phrenic artery. *d*, small artery to the adrenal gland. *e*, adrenolumbar artery. *f*, adrenolumbar vein. *g*, postcava. *h*, right internal spermatic artery. *i*, left phrenic artery. *j*, left adrenolumbar vein. *k*, left adrenolumbar artery. *l*, left renal vein. *m*, left renal artery. *n*, left kidney. *o*, left spermatic vein. *p*, left ureter. *r*, inferior mesenteric artery. *s*, dorsal aorta.

the increase in the volume of the graduate's contents. Cat 1 weighed 2350 grams; the volume of its only kidney was 27 cc. The weight of cat 2 was 2280 grams, and the volume of each kidney was 12 cc. The two cats weighed nearly the same, yet

the kidney of cat 1, having about twice as much work to do as either kidney of cat 2, was about twice as large as the latter. Figure 2 shows the relative sizes of the kidneys from these two animals.

The body length of cat 1 was from 3 to $3\frac{1}{2}$ cm. *less* than the body lengths of cats 3, 4 and 5, yet one kidney from each of the last three measured 15, 21 and 9 cc., respectively. These measurements clearly demonstrate the hypertrophied condition of the kidney in cat 1.

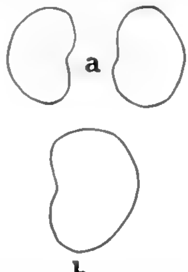

Fig. 2 *a*, the two kidneys from cat 2. *b*, the single kidney from cat 1.

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Resumido por el autor, Adolf H. Schultz.

Observaciones sobre el canalis basilaris chordae.

En los huesos basioccipitales de dos adultos de raza blanca, ha encontrado el autor un canalis basilaris chordae completo, resto de la parte craneal de la cuerda dorsal, y el mismo canal, parcialmente obliterado, en el cráneo de un filipino adulto. También ha encontrado la misma estructura en los cráneos de un niño blanco y cuatro niños negros. En uno de los casos observados en el adulto, la anomalía citada coincide con un os Incae, y en uno de los niños con un canal cranio-faríngeo.

Translation by Dr. José Nonidez,
Columbia University.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, NOVEMBER 18

OBSERVATIONS ON THE CANALIS BASILARIS CHORDAE

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THREE FIGURES

Out of twenty-two adult human skulls[1] examined by the writer, a complete canalis basilaris chordae seu medianus, which perforates the basioccipital bone in a sagittal direction, was found in two whites, and the same canal, partially closed, in one Filipino. In a material consisting of thirty-eight skulls (twenty-six negro and twelve white) of fetuses and infants, ranging in age from the eighth month of prenatal to the second month of postnatal life,[2] the canalis basilaris, represented only by its posterior part, was found in four negroes and one white.

The canal was first described by Gruber in 1880, since which time a number of new cases of more or less complete canalis basilaris have been reported by Romiti ('81), Fusari ('89), Staderini ('00), Paravicini ('03), Le Double ('03), and Perna ('06). The last-mentioned writer states that it occurs in 2.47 per cent of adults and 4.22 per cent of children. The greater frequency in children would indicate that the canal may become obliterated during the process of growth, but its remaining patent is not necessarily influenced by age, inasmuch as the author found it complete in a woman about 75 years of age.

The canalis basilaris has been explained as a trace of the cranial part of the chorda dorsalis, which normally disappears after or during the third month of intra-uterine life. The similarity between the course of the chorda in the cranial base

[1] This material belongs to the Anatomical Department of the Johns Hopkins Medical School. I wish to thank Dr. W. H. Lewis for his kind permission to utilize the same.

[2] Belonging to the Carnegie Laboratory of Embryology.

of fetuses and that of the canal in adults (fig. 1) is so striking as to make this theory appear more than probable.[3] In fetuses the chorda dorsalis, emerging from the dens epistrophei, enters the basioccipital plate on its dorsal side, extends forward and downward, running for some distance beneath the base of the cranium between the latter and the dorsal wall of the pharnyx, after which it again enters the skeletal tissue to extend up toward the dorsum sellae turcicae. A detailed description of these conditions is given by Huber ('12) in his excellent paper on the chorda dorsalis and pharyngeal fossa. The relative distances from the points where the chorda enters and where it leaves the

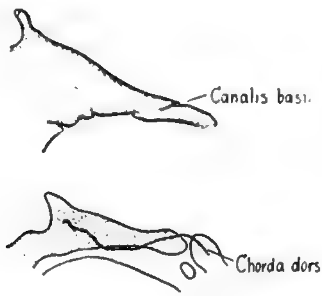

Fig. 1 Median sagittal section through the basioccipital and basisphenoid bone of an adult Filipino with incomplete canalis basilaris, and through the corresponding region of the head of a 10-weeks fetus. (Lower drawing is schematic.)

basilar part of the occipitale to the anterior border of the foramen magnum may vary in different fetuses, just as the location of the terminal points of the canalis basilaris may differ slightly in individual cases, although they are found always in the midsagittal plane.

The canalis basilaris chordae was found to be widest and most typical in the skull of a white man approximately 40 years of age (fig. 2). In this case the posterior opening of the canal is 3 mm. in diameter, its narrowest width 0.9 mm., and its length 19 mm. Its anterior end opens into a fossa pharyngea. Just

[3] Foramina nutritia may occur at about the same place; therefore care must be taken not to diagnose this condition as incomplete canalis basilaris.

behind this opening is a well-developed tuberculum pharyngeum. Canales condyloidei are absent.

In the skull of a white woman 75 years of age the canal allows the passage of only a bristle 0.6 mm. thick (fig. 3). The length of the canal is 13 mm. No tuberculum or fossa pharyngeal were found. On each side there is a wide canalis condyloideus.

The canalis basilaris in the skull of a Filipino approximately 30 years of age is partially obliterated (fig. 1, upper drawing). The small inner opening is 6 mm., and the ventral opening 17 mm. from the anterior border of the foramen occipitale. The canal is patent except for a distance of 3 mm. in its middle portion. Seven millimeters anterior to the ventral opening there is another median foramen, pointing for a short distance in the direction of the pituitary fossa. A shallow furrow combining the two ventral foramina makes it certain that the most anterior foramen is a continuation of the canalis basilaris. A tuberculum pharyngeum is present in this case, but only slightly developed; there is also a wide canalis condyloideus on each side. It may be mentioned that this skull shows another anomaly, i.e., a persistent sutura occipitalis transversa, which forms an os Incae verum, which changes the form and size of the occiput and the entire cranium. These changes were demonstrated by the author ('15) on fourteen skulls showing the same anomaly. In the three skulls described herein the posterior border of the foramen occipitale is thick, slightly elevated and forms a discernable arch—a manifestation of an occipital vertebra, which Kollman ('07) sees also in the canalis basilaris.

As mentioned above, the posterior end of the canalis basilaris was found in five skulls of infants and fetuses. One of these is especially interesting, because combined with it was the likewise very rare persistence of a canalis cranio-pharyngeus. This combination was found also by Perna on the skull of a young Tuscan. These two abnormal canals have several features in common. They are both remnants of embryological structures which normally disappear during the first half of intra-uterine life; both occur more frequently in children than in adults, and both are often found in an incomplete—that is, a partially

obliterated, state. As the explanation of atavism was denied by the author ('16) in the case of the canalis cranio-pharyngeus, he likewise does not consider it probable that the canalis basilaris chordae is a pure atavism. It is much more likely to occur in consequence of the coincidence of abnormal early or rapid ossification of the basioccipitale and the late disappearance of the chorda dorsalis. However, many more cases of the presence of the canalis basilaris in man, where it is known to give rise to tumors, and also in other mammals[4] will have to be reported before definite conclusions can be reached as to whether it plays a phylogenetic rôle.

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[4] The author found the canal in a few skulls of guinea-pigs; Fusari ('91) found it in the skulls of a colt and a calf.

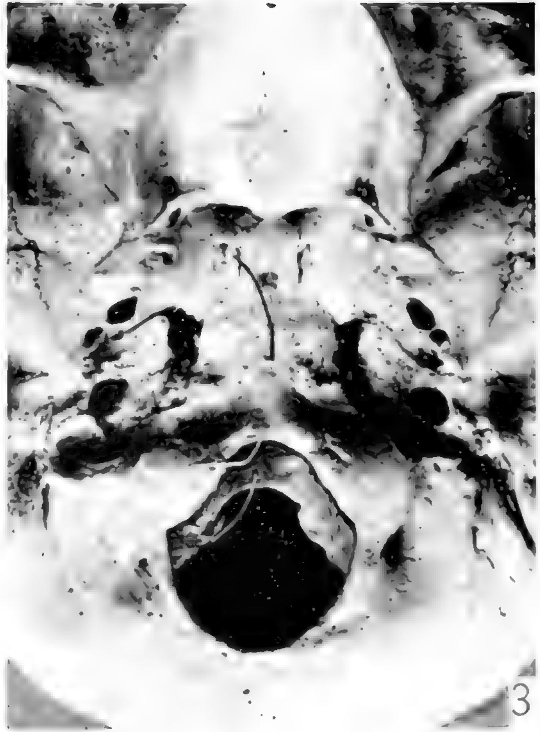

Fig. 2 Base of skull of a white man with canalis basilaris through which a piece of wire has been passed. A mirror was placed in the foramen occipitale to show the inner dorsal opening of the canal.

Fig. 3 Base of skull of a white woman with canalis basilaris. Mirror in foramen occipitale shows posterior opening.

Resumido por los autores, Eleanor L. y Eliot R. Clark.

Sobre las reacciones de ciertas células de la cola del renacuajo bajo la acción de los colorantes vitales.

En el transcurso de las observaciones sobre el crecimiento y capacidad de reacción de las células y tejidos de la porción transparente de la cola del renacuajo, los autores han creido conveniente estudiar los efectos de la coloración vital sobre estas células. Los renacuajos fueron colocados en soluciones de diversos colorantes y después de teñidos fueron observados en un micro-acuario, anestesiados con cloretona. Han empleado casi exclusivamente tres colorantes, el rojo neutro, pardo de Bismarck y el azul trypan, a causa de la semejanza de su acción y por teñir todos ellos el endotelio linfático con especial claridad. Los más difusibles de estos colorantes rojo neutro y pardo de Bismarck tiñen las células más rápidamente que el azul trypan, colorante coloidal, coloreando pequeños gránulos regulares, preformados en apariencia, existentes en las células epidérmicas. Además, el rojo neutro tiñe con brillantez el contenido de un sistema subepidérmico ricamente ramificado. El azul trypan no tiñe la epidermis. Los tres colorantes tiñen un gránulo no constante, probablemente preformado, existente en las paredes de los vasos sanguíneos. Tanto el rojo neutro como el pardo de Bismarck y el azul trypan se depositan en forma de acúmulos granulares tintóreos en las áreas perinucleares de los linfáticos, en ciertas células emigrantes y leucocitos y en los apéndices de las células mesenquimatosas. Las relaciones fisiológicas que la reacción hacia estos colorantes vitales pone de manifiesto en estos diferentes tipos de células, se debe probablemente a la propiedad fagocítica común a todas ellas.

Translation by Dr. José Nonidez,
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AUTHORS' ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, NOVEMBER 18

ON THE REACTION OF CERTAIN CELLS IN THE TADPOLE'S TAIL TOWARD VITAL DYES[1]

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TWO FIGURES

In the course of studies on the growth and reactive powers of living lymphatic and blood-vessel endothelium, mesenchyme cells, and wandering cells in the tadpole's tail, it seemed advisable to try the effect of various vital dyes on these cells and tissues. The following experiments were started with the twofold object of determining which vital dyes can best serve as aids in the microscopic study of these cells and of throwing more light on the nature of these cells through a knowledge of their response to the various vital dyes. Needless to say, a region such as the transparent tail of Amphibian larvae, where all the cells can be observed in detail, in the living animal, is an advantageous place to study the effects of vital staining.

Ehrlich ('94) used the tadpole in making his first test of neutral red as a vital stain. He mentions briefly that tadpoles are stained very intensely after remaining for a day in a 1 to 10,000 to a 1 to 100,000 solution of the dye.

Arnold ('00) and Fischel ('01) stained Amphibia with neutral red and methylen blue, but neither of them gave a description of the effect of vital staining on the cells of the transparent tails of the larvae.

One of the authors (E. R. Clark, '09) used neutral red in his studies on the growth of living lymphatic capillaries in the tadpole's tail, and noted that the granular areas surrounding the nuclei took up the stain.

Wislocki ('16, '17), in his valuable studies on the action of the

[1] Accepted for publication January, 1918.

acid azo dye, trypan blue, on Amphibian larvae and on teleosts, makes the interesting observation that trypan blue stains the perinuclear areas of the lymphatic endothelium brilliantly and specifically.

The points of similarity between Wislocki's description of the staining of lymphatic endothelium with trypan blue and the observations of their appearance after staining with neutral red, made it seem worth while to compare the results of using these two stains and to study the effect of various other vital dyes on the cells of the tadpole's tail.

The tadpoles used for these experiments were chiefly larvae of Rana pipiens. The method of chloretone anaesthesia and observation in the upright chamber, devised and previously described by one of the authors (E. R. Clark, (09, '12) were employed. The vital stains used were neutral red, Bismarck brown, trypan blue, gentian violet, and methylen blue.

NEUTRAL RED

Neutral red, a basic dye soluble in lipoids and readily diffusible, was first used by Ehrlich ('94) and has been used by many investigators, perhaps most extensively by Fischel ('01). He found that salamander larvae, stained for several hours in neutral red and then transferred to fresh water, retained their red color for as long as eleven months. Fischel found that this dye continues to be absorbed, and that animals left for a long time in the dye solution become so densely stained as to be almost black.

In the present experiments, tadpoles were placed in neutral red solutions of dilutions varying from 1 to 5000 to 1 to 200,000. The cumulative effect of the dye can best be appreciated when it is stated that the larvae left overnight in a 1 to 200,000 solution were more intensely stained than those left for an hour in a stain of 1 to 5000. The best results for the study of the cells in the subcutaneous tissue of the tail were obtained when the tadpoles were allowed to remain for one or two hours in a 1 to 10,000 solution.

The progress of the stain can best be watched by placing a tadpole in a 1 to 10,000 solution of neutral red for five to twenty minutes and then removing it to a chloretone solution (in the observation chamber), for the stain continues to penetrate the tail after the animal has been removed from the dye. The stain appears first as small granules in the cells of the epidermis, as described by Arnold ('00) and Fischel ('01), and as large round bodies in an irregular branching system immediately beneath the epidermis. After two hours of observation, with occasional additions of minute quantities of the stain, the epidermis and this subepidermal system become brilliantly red while the subcutaneous structures are still unstained. Next, the perinuclear areas of the lymphatic vessels and certain large wandering cells take up the stain in the form of numerous granules. Two hours later, a few isolated red granules are visible in the endothelial wall of some of the blood capillaries. A day or two later, red granules make their appearance on the processes of the connective-tissue cells.

After the stained larvae have been removed to fresh water, the red stain in the subepidermal system gradually changes to orange and, at the end of a week or two, fades to a pale yellow. However, the neutral red remains in the other stained cells and tissues for more than a month (as long as the tadpoles were kept under observation) with no diminution in its intensity.

The cells of the epidermis, after staining with neutral red, contain red granules of uniform size, regular contours, and of fairly even distribution. These stained granules have been figured by Arnold ('00) and by Fischel ('01). No stain was ever found in the nuclei.

The peculiar system in the subepidermal region, which shows up so strikingly with neutral red, was observed in 1908–09 by one of the authors, and, curiously enough, appears not to have been described by any of the numerous investigators who have made use of this dye in the study of Amphibian material.

This subepidermal system is made up of a series of large, centrally placed, irregularly shaped nuclei, from which radiate hollow-branched protoplasmic processes of a delicacy so extreme

that they can be followed only with the greatest difficulty. These hollow processes are divided into small segments by delicate partitions, and contain fluid, as was shown by the fact that now and then a pigment granule was observed in one of the compartments, in active Brownian movement. Such granules were not seen to migrate from one compartment to another. These structures lie immediately under the epidermis, and are found generally over the body and tail. In the tail, they are rather sparsely distributed near the margins of the fin and the tip of the tail, increasing in number toward and over the central muscular portion. They have been observed in a number of species of toad and frog larvae, the pattern differing in different species. The system shows most strikingly in young Hyla pickeringii larvae. Its history in later stages and in frogs has not yet been followed.

When first treated with neutral red, the fluid contents of the branched processes are deeply and uniformly stained. Soon, however, the uniformity is lost, for some compartments lose their stain entirely, while in others the stain becomes more dense. After a day or two, the processes have the appearance of irregular strings of pink beads, since the walls of intervening, unstained compartments collapse and are seen only with great difficulty. A casual observation at this stage shows what might be described as a 'freckled' appearance, since the compartments form spheres of different sizes, apparently isolated. The stain gradually fades out from this system and, after a few days, has disappeared entirely. It was not stained by any of the other dyes used.

The lymphatic endothelium is brilliantly stained with neutral red and the dye is lodged in the area around the nucleus. One of the authors, E. R. Clark, '09, '12) has given a detailed description of the appearance and behavior of these granular or nuclear areas in living lymphatic capillaries. In the unstained tadpoles he observed that the nuclear and perinuclear areas merge imperceptibly into one another, and he showed the relationship of the two by first drawing the lymphatic in the living and then drawing the same vessel after fixation and staining. The stain with neutral red differentiates the two portions of the granular

areas—the nuclear and perinuclear—in the living tadpole, since the nucleus, which fails to stain, then shows up as a clear lens-shaped region surrounded by a reddish area containing many deeply stained granules. The stain is confined to this area around the nucleus, the rest of the cytoplasm remaining clear (fig. 1, *A*). The stained granules vary in size and shape, some of them are large and refractile, others small and dark. Black and brown granules can be seen between the red ones. If a stronger stain is used, the red granules are found to be more numerous as well as larger and darker in color, and they frequently occur in irregular clumps and masses.

Not all of the wandering cells are stained with neutral red. Here and there in the tail, large round cells, containing brilliantly stained red granules, can be seen (fig. 1, *C*). These cells often contain black pigment as well. Small wandering cells, as a rule, contain no stain.

A day or two after removal to fresh water, in tadpoles stained for one or two hours in a 1 to 10,000 solution, the mesenchyme cells begin to show traces of stain. The dye is present as small granules occurring singly on the cell processes. Occasionally a red halo can be found around the black pigment spot present in many of these cells. With a more intense stain the red granules in the mesenchyme cells make their appearance much sooner, and they are then more numerous as well as darker in color and may be seen in the cell bodies clustered about the base of the processes as well as along the processes themselves (fig. 1, *B*).

In tadpoles stained with neutral red, a few red granules may occasionally be found in the endothelial cells of the blood vessels. Such granules are small and round and occur close to the nuclei, only one or two to each nucleus. The difference in the appearance of the practically unstained blood capillaries and the lymphatics with their bright red, coarsely granular patches is very striking.

No stain was found in the nerves of the tadpole's tail nor in any of the cells inside the blood vessels or lymphatics. With the exception of the branching system beneath the skin, which

appears to be in a class by itself, the living cells of the tadpole's tail can be divided into three classes with respect to their manner of staining with neutral red:

1. Cells which show no granules stained with neutral red. These are nerve cells, blood cells inside the vessels, and some of the wandering cells.

2. Cells which contain small red granules, regular in shape and of fairly even distribution, after staining with neutral red. These are the epidermal cells and the endothelial cells of the blood vessels. An increase in the strength of the stain has no effect on the size, shape, or number of the stained granules in this type of cell.

3. Cells which show large accumulations of red granules after staining with neutral red. These are the endothelial cells of the lymphatics, certain large wandering cells, and the stellate connective-tissue cells.

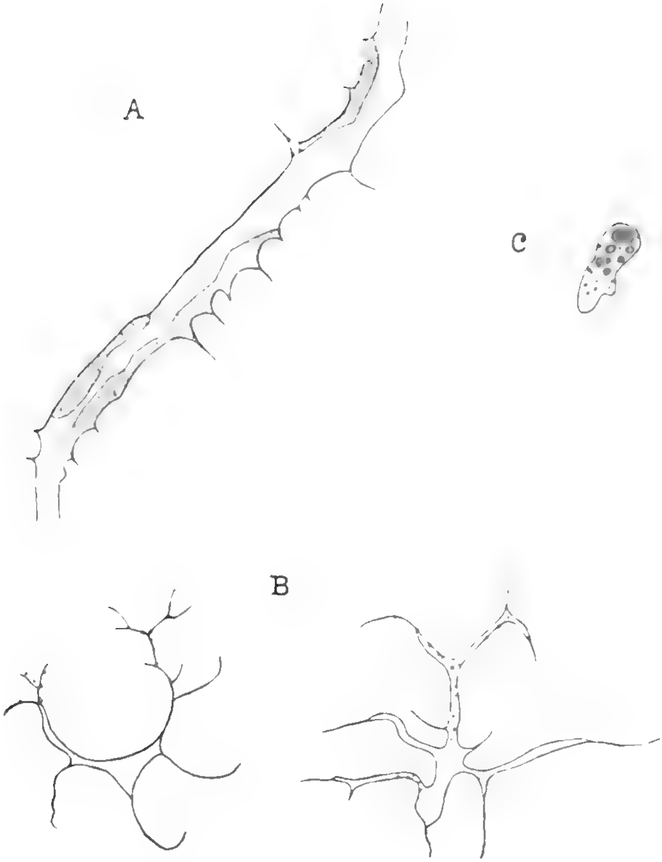
With these three types of cells, the neutral red granules increase in number and size with an increase in the strength of the stain or merely with the passage of time after the larva has been transferred to fresh water.

BISMARCK BROWN

Bismarck brown is a well-known basic azo stain, soluble in lipoids, which has frequently been used as a vital dye. In the present experiments, Bismarck brown was found to be very similar to neutral red in its action, although it stained somewhat

Fig. 1 Sketches from the tail of a living frog larva (Rana pipiens) which had been stained with neutral red, 1 to 10,000, for two hours, and then transferred to fresh water. The sketches were made three days later. *A*, Lymphatic capillary. The perinuclear areas contained red in the form of granules. *B*, Mesenchyme cells with red granules in certain of the processes. *C*, Wandering cell containing red granules and red 'bodies,' of various sizes. Enlargement = approximately × 740.

Fig. 2 Sketches from the tail of a living larva, stained with trypan blue, 1 to 1600, for four days. Sketch made a few minutes after transferring to chloretone solution. *A*, Lymphatic capillary with blue granular stain in the perinuclear areas. *B*, Mesenchyme cells with blue granules on many of the processes. *C*, Wandering cells containing blue granules of various sizes. Enlargement = × 740.



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less intensely than a neutral red solution of the same strength. The clearest pictures were obtained by placing a tadpole in a 1 to 10,000 solution of the dye, leaving it there for three or four hours and then removing it to fresh water. As in the case of neutral red, tadpoles stained overnight in a 1 to 200,000 solution were much more deeply stained.

Like neutral red, this stain penetrates the skin rapidly and, after a few minutes, is visible in the epidermal cells in the form of round granules, usually one to each cell. In the region beneath the epidermis, there is no trace of the system which shows up after neutral red, but here the stain is deposited as numerous rods and needles. Fischel ('01) has figured these structures, both inside of cells and between them, and does not hesitate to call them crystals. One-half to one hour later, cells in the anterior of the tail fin begin to take up the stain. The perinuclear areas of the lymphatics and some of the large wandering cells first show the dye in large amounts, and a few brown granules may be detected in the walls of some of the blood vessels. A day after a tadpole, stained in this way, has been transferred to fresh water, brown granules can be observed on the processes of the mesenchyme cells. The crystals in the epidermis fade and disappear after a day in fresh water. On the other hand, the stained granules in the epidermal cells remain and those in the lymphatic endothelium, in the wandering cells, and in the connective-tissue cells become more numerous and prominent.

As in the case of neutral red, lymphatics stained with Bismarck brown show the dye only, in the area around the nucleus. The stain is present as brown or black granules of different sizes and the space between the granules is colored a yellowish brown. The wall of the lymphatic, in the neighborhood of these stained areas, stands out more distinctly than the rest of the endothelium, as if it had been outlined with pen and ink. With a very faint stain, obtained by leaving the tadpole for half an hour in the dye solution, this outlining of the lymphatic wall and a very few brown granules in the perinuclear areas are the only effects of the staining. With a 1 to 5000 concentration of the dye or after staining for several hours in a weaker solution, the granules

in the perinuclear areas of the lymphatic become larger and more numerous and often appear in clumps.

The staining of the large wandering cells is similar to that described for neutral red. But, in addition to the small brown granules, large refractile globules with regular outlines which took a scarlet or brown stain, were present in some of the wandering cells.

No stained granules were observed in the nerve cells nor in the blood cells inside the vessels. It will be seen from the above description that, aside from the characteristic deposit of crystals in the subepidermal region and the failure to stain the branching system in this region, Bismarck brown stains the same structures as neutral red and in a practically identical manner.

TRYPAN BLUE

Trypan blue is an acid azo dye belonging to the group of benzidine dyes containing trypan red and pyrrhol blue, which have aroused so much interest recently because of their action as vital stains. Trypan red, first used by Ehrlich as a cure for trypanisomiasis, was discovered incidentally to be a true vital stain for the tissues of the host. Nicolle and Mesnil ('06) discovered trypan blue and found it to be equally effective, and Bouffard ('06) investigated its staining properties. Later, Goldmann ('09) made a more extensive study of the effects of staining with pyrrhol blue and described various cells which stained with the dye and to which he gave the name 'pyrrhol cells.' Evans and Schulemann ('14, '15) used trypan blue chiefly for their investigations of vital staining. All of these workers used mammalian material for their studies.

Wislocki ('16, '17), in studying the effect of trypan blue on amphibians and fish, found, in addition to cells corresponding to those of mammals in their ability to store this dye—namely, the Kupffer cells of the liver, groups of mononuclear cells in the mesentery and omentum, and the cells of the convoluted tubules of the kidney—a marked vital staining of the epithelium of the gills, which he considered to be an evidence of an excretory action, and a staining of the lining and contents of the alimentary

canal, which appeared to represent the place of absorption of the dye. And, in addition, he describes the lymphatic system as staining in its entirety and in a brilliant manner with trypan blue. In the tail of the tadpole, where liver, alimentary canal, kidney, and gills are all absent, he states that trypan blue is a specific vital stain for the lymphatic endothelium, since no other cells show a trace of the dye. He states that the dye is present in the form of granules in the perinuclear areas of the lymphatics.

In the present experiments, tadpoles, some of them newly hatched and others two or three weeks old, were placed in solutions of trypan blue in tap water. A dilution of 1 to 1600 was the one most frequently employed, this being the average of the various strengths recommended by Wislocki. In contrast to neutral red and Bismarck brown, this dye is absorbed very slowly, tadpoles showing no trace of the dye after remaining twenty-four hours in the stain. At the end of the second day, a blue stain was visible in the lymphatic endothelium, and this became more marked with every day that the tadpole remained in the stain, until, at the end of a week, the lymphatics were vividly blue, as described by Wislocki.

If a tadpole is removed to fresh water after a week in the dye solution, the vital stain is not only retained by the cells which have taken it up, but, as in the case of neutral red, it becomes even more intense. In fact, the most rapid staining with trypan blue was obtained by placing a larva overnight in a 1 to 600 solution and then transferring it to fresh water. Six or seven hours later, a definite stain in the lymphatics of the tail could be detected.

No staining of the epidermal cells takes place with trypan blue. As already stated, the perinuclear areas of the lymphatic take the stain, and the blue color is present in the form of granules of different sizes and a pale blue tinge between the granules. In addition, black and brown pigment granules are also present (fig. 2, A). The stained areas resemble closely those present after staining with neutral red and Bismarck brown. In order to test this similarity of action, a tadpole which had been stained for a week in trypan blue, was selected and a lymphatic capillary drawn with the camera lucida. The larva was then placed in a

solution of neutral red, 1 to 5000, for twenty minutes, and again placed in the observation chamber and the same region located and the lymphatic again drawn. The neutral red had replaced the blue stain, and the red areas corresponded exactly in size, shape, and position to those previously drawn.

In addition to the deep stain of the lymphatic endothelium, some of the large wandering cells in the subcutaneous tissue of the tail were observed to contain an abundance of blue granules (fig. 2, *C*). These cells are similar to those which stain with neutral red and Bismarck brown. The stained wandering cells can be seen two or three days after placing a tadpole in the dye solution, or as soon as the stain becomes visible in the lymphatics.

After four to six days in a solution of this strength, small blue granules make their appearance on the processes of the mesenchyme cells (fig. 2, *B*). At first such granules are somewhat pale and hard to identify without the aid of the oil-immersion lens. A few days later, they become darker and they continue to increase in number and in intensity of staining, so that, after one or two weeks in the dye solution, these blue granules in the mesenchyme cells are visible with the low power of the microscope and are as conspicuous as those present after staining with neutral red. The distribution of trypan blue granules is the same as in the case of the other two dyes, namely, on the cell processes and in the cell body near the base of the processes.

A few small but undeniably blue granules were noticed near the nuclei of some of the blood vessels.

No blue stain was observed in nerves nor in the blood cells inside the vessels. There were always a number of wandering cells present in the tissue spaces which failed to stain.

Aside from its slower action and its failure to stain any of the cells of the epidermis, trypan blue was found to stain the same structures and in the same manner as neutral red and Bismarck brown.

The total lack of stain in the epidermis permits one to obtain a clearer picture of the deeper structures and, for this reason, trypan blue is the most satisfactory of these three stains as an

aid in the study of living lymphatics. It has also the great advantage that it can be preserved after fixation and the effect of the staining studied in permanent preparations. However, if, as frequently happens, a rapid stain of the living lymphatic is desired, it is useful to know that a similar result can be obtained within a few minutes by placing the tadpole in a solution of neutral red or Bismarck brown. The present observations show that trypan blue is not a specific stain for the lymphatics of the tadpole's tail, since some of the wandering cells and all of the mesenchyme cells were found to possess the power of storing the dye. For this reason it can scarcely be used as a means of distinguishing the origin of different types of cells.

The relatively slow action of trypan blue, together with the fact that none of the stain is deposited in the epidermis, would lead one, on theoretical grounds, to accept Wislocki's hypothesis that the dye is absorbed through the alimentary canal. If this were true, the dye would necessarily reach the tail through the general circulation, as in the case of injections of dye into the peritoneal cavity. To test this point, the blood hearts were removed in a number of newly hatched tadpoles before the circulation had commenced, and, a day later, the larvae were placed in a 1 to 1600 solution of the dye. Three days later, the lymphatics and some of the large wandering cells showed the typical collection of blue granules and, a few days afterward, blue granules appeared in the connective-tissue cells. The stain appeared as soon as in the control specimens, was equally intense, and was present in the same locations. Therefore, the conclusion seems warranted that trypan blue penetrates the skin of Amphibian larvae, as do neutral red and Bismarck brown, and that, in all probability, its slower action is due to its lower rate of diffusion.

METACHROMATIC STAINING WITH TRYPAN BLUE

Von Möllendorf ('15) has shown, by dialysis experiments, that trypan blue is composed of two dyes—a red substance which is highly diffusible and a blue substance which is less diffusible

and ordinarily masks the red element. Schulemann ('15) and Evans and Schulemann ('15) have described a metachromatic staining with certain vital dyes, among them Congo rubin, in which red vacuoles were observed to contain blue granules. Schulemann found by experimentation that Congo rubin would change to blue either with the addition of acid or with an alteration in the electrolytes, caused by adding certain salts. He concluded that the second explanation, namely, a change in the physical state of the dye, was the true one for this phenomenon of metachromatic staining.

In the course of the present experiments a number of cases of metachromatic staining with trypan blue were encountered and we were unable to prevent or to produce this type of staining at will. The first example was noted in a tadpole which had remained in a 1 to 1600 solution of trypan blue for six days. On the third day in the stain, the lymphatic endothelium and certain wandering cells were seen to contain blue granules, and on the following two days the stain became a more brilliant blue. But, three days later, when this tadpole was observed under the microscope, the perinuclear areas of the lymphatic and the stained wandering cells were seen to contain pink granules as well as purple and blue ones. During the next few days the red stain became more conspicuous and finally replaced the blue entirely. In this specimen, the stained granules in the mesenchyme cells were red when first noted.

After this peculiar experience, particular pains were taken with the cleanliness of dishes, and dye solutions were watched with especial care to prevent any accidental contamination. However, some days later, another tadpole, which had been placed in an 'old' dye solution (one made up a week previously) showed red coloration of lymphatics and wandering cells after three days in the stain, or as soon as any stain became visible. As in the former case, the gills, alimentary canal, and certain injured areas in the tail, where a diffuse stain was present, all remained blue. For some time we believe that the fact that the dye solution had been allowed to stand so long in this case was the reason for the change in color, but a week later two more

tadpoles, which had been living in a freshly made up solution, showed a violet coloration of their lymphatics, which changed to pink soon after removal of the larvae to fresh water. During all this time, many tadpoles had been examined which showed the typical blue coloration of lymphatics, wandering cells, and mesenchyme cells.

We next tried to discover whether the tap water with which the dye solutions were made up was responsible for this change in color. This seemed a plausible explanation for this recurring phenomenon, which Wislocki had not encountered in his experiments with tadpoles, in view of the high salt content of the Missouri water. For this reason, we proceeded to make up the stain for half the experiments with rain water, collected after a hard rain so that it was free from soot or dirt of any kind, using tap water for the others, and observed the two sets of tadpoles from day to day. The tadpoles from the rainwater solutions were observed in chloretone made up with distilled water. For more than a month, every tadpole stained in the rain-water solutions of trypan blue showed the typical bright blue stain in the lymphatics wandering cells, and connective-tissue cells of the tail, while those from the tap water, on a few occasions, showed a navy blue, purple, or red stain in these same cells. Just as we were convinced that we had discovered the cause of this change in the usual mode of staining, we found three tadpoles, which had been placed three days before in a fresh solution of trypan blue in rain water and which had been carefully protected from contamination, which showed red granules in the perinuclear areas of the lymphatics and in the large wandering cells. Also, at about the same time, a tadpole placed in an 'old' solution of trypan blue in tap water, which had previously caused a red stain to appear in two other larvae, showed only blue granules in lymphatics, wandering cells, and mesenchyme cells. The explanation for this occasional change in the staining properties of trypan blue or in the behavior of certain tadpoles toward the dye remained undiscovered and the phenomenon could not be produced at will.

In the cases in which red staining resulted from the use of trypan blue, the picture presented resembled closely that seen when blue staining was obtained, save for the difference in color. Thus, no stain appeared for two or three days; then the lymphatic endothelium and some of the wandering cells showed the red color in the form of granules and, a few days later, red granules made their appearance on the processes of the connective-tissue cells (fig. 4). The granules in the lymphatic endothelium, however, were often finer than those which were present in the case of the typical blue staining or after the use of neutral red. In the wandering cells, blue and purple granules were often visible in addition to the red ones. In the mesenchyme cells, a red halo around the black pigment spot often showed before any stain appeared elsewhere. The red granules on the processes of the mesenchyme cells were definitely noticeable a day or two earlier than in the case of the blue stain. This was probably due to the greater ease with which red granules can be distinguished from the unstained elements of living cells. As with the blue stain, an occasional stained granule was observed in the walls of some of the blood vessels. The only marked difference in the case of the tadpoles showing this metachromatic staining was noticed in some of the superficial pigment cells which were seen on several occasions to contain a few red granules. This was never noted in larvae staining normally with trypan blue.

VITAL STAINING AND INJECTED FAT

In a former paper (E. R. and E. L. Clark, '17), we showed that small globules of fat injected into the subcutaneous tissue of the tadpole's tail were absorbed through the activity of leucocytes and lymphatics. We also mentioned that, in such tadpoles which had been stained with neutral red, the stained leucocytes in the vicinity of the old globules were especially conspicuous.

This experiment of injecting fat into the transparent tail fin and then staining the tadpole intra vitam was repeated. The two stains used were neutral red and trypan blue. Some of the large

wandering cells which chanced to be in the neighborhood of the olive oil at the time of injection moved toward the globule and flattened out on its surface. Numerous leucocytes, migrating from near-by blood vessels, crowded around the periphery of the fat. All of these cells surrounding the olive oil became pigmented with finely divided fat, and most of them also became loaded with the coloring matter, red or blue as the case might be. As in the normal specimens, the lymphatic endothelium and certain large wandering cells scattered at other points in the tail, took up the stain brilliantly. With both neutral red and trypan blue, certain of the migrated leucocytes which had reached the oil globule and which there proceeded to ingest the fat in the form of fine brown pigment granules, never showed the slightest trace of the dye. Also, certain wandering cells, present in the tissue near the site of injection, were not attracted toward the olive oil and showed neither brown pigment nor stain. These cells, which surrounded the oil globule and took part in the fat absorption, when stained with gentian violet, a nuclear stain, were all shown to possess a single round nucleus.

Anitschkow ('14) has shown that the Kupffer cells of the liver, the reticulo-endothelial cells of the spleen, bone marrow, and lymph glands, and the macrophages of the connective tissue—those cells which are known to possess the power of storing colloidal metals and colloidal vital dyes also have the power of cholesterin fixation after feeding with fat. On the other hand, Fiessinger and Marie ('09) have demonstrated lipase in the lymphocytes. The present observations show that many of the cells which take up injected fat also stain brilliantly with vital dyes. In this instance, the colloidal dye, trypan blue, and the more highly diffusible neutral red produce identical results. In addition, certain other leucocytes (possibly lymphocytes) which are also attracted toward the fat, do not stain with these dyes. And still other wandering cells show no reaction either toward the fat or the dyes.

The three dyes, neutral red, Bismarck brown, and trypan blue, were used almost exclusively in these observations, because they proved to be true vital dyes, because of the similarity of their

action and because they stained the lymphatic endothelium with especial distinctness. The other stains tried did not prove so satisfactory from these points of view, and the result of their use will be reported very briefly.

GENTIAN VIOLET

Russell ('14) reports that this anilin dye, which is effectual in killing bacteria in strengths of 1 to 1,000,000, at the same time proved to be a true vital stain for frog tissue, in cultures, staining the whole culture and the nuclei in particular and not preventing further growth of the tissue He found that cultures made from adult frog tissue would grow and show the stain in dilutions of 1 to 1000 up to 1 to 20,000.

Tadpoles placed for an hour in gentian violet—1 to 10,000—were violet in color and, on examining such a larva in the observation chamber, all the cells of the tail were found to be beautifully stained, the cytoplasm a pale lavender tinge and the nuclei a deeper violet. In addition to this uniform lavender color, many small black granules stood out clearly in the cytoplasm. Active Brownian movement of these granules could be observed with the oil-immersion lens. However, we were unable to obtain a true vital stain for the cells of the tadpole's tail with this dye, for larvae stained in this manner were invariably dead and macerated on the following day. Staining for one hour in dilutions of 1 to 20,000, in which case the resulting stain was much fainter, also proved fatal. Tadpoles, stained for fifteen minutes in a 1 to 20,000 solution, survived for over twenty-four hours, but showed only the faintest trace of color. Strengths of 1 to 50,000 and 1 to 100,000 for one or two hours were tried, but the tadpoles failed to show any stain and they lived for only a few days afterward.

Although proving toxic in strengths necessary for obtaining a satisfactory stain, gentian violet proved to be of value as a nuclear stain in those cases in which it was possible to sacrifice the tadpole. The use of gentian violet to demonstrate the character of the nuclei of those leucocytes which collect around the globules of injected fat has been mentioned.

METHYLEN BLUE

This dye, which Ehrlich ('86) showed to be a vital stain for the nerves of the frog, has been used by many investigators. In addition to its especial affinity for nerves, methylen blue has been described (Fischel, '01) as staining granules in many cells, especially the pigment cells in the skin of Amphibia, certain granules in the epidermal cells, and the mucin content of the Leydig cells. Arnold ('99) mentions blue granules in nerve cells, mast cells, and leucocytes after staining Amphibian tissues with this dye. Ehrlich, Arnold, and Fischel all obtained double staining effects with methylen blue and neutral red, certain granules staining with one dye and others with the other.

Possibly the strengths of this stain tried in the present experiments were too weak, but no very striking results were obtained by the use of methylen blue as a vital stain for the tails of tadpoles. After one hour in a 1 to 10,000 solution of the dye, certain of the large superficial pigment cells of the tail showed a greenish-blue coloration of their granules. This staining effect has been described by Fischel. No stain was noted in any other type of cell with the exception of certain small leucocytes within the blood vessels, which were stained a bright diffuse blue. Possibly better results could be obtained by more extensive investigation.

DISCUSSION

It is obviously difficult to formulate any one theory of vital staining which will adequately explain the action of all vital dyes. Thus, Fischel's ('01) statement that only the basic dyes are taken up by living tissue is contradicted by the striking results obtained by staining in vivo with pyrrhol blue, trypan blue, and other acid benzidine dyes. The lipoid theory of vital staining, brought forward by Overton ('00), has also been shown to be inadequate as a general explanation, in view of the action of this same class of dyes, all of which are insoluble in lipoids. And the assertion of many authors that diffuse staining and nuclear staining are always evidence of the death of the cell so stained or at

least of injury to it, has been shown to be untrue in the case of gentian violet, which Russell ('14) has shown to be capable of acting as both a diffuse protoplasmic and a nuclear stain.

At the present time, the two chief theories dealing with the action of vital dyes are: 1) The chemical theory, advanced by Ehrlich ('04, '09), that vital staining is evidence of a true chemical union between some part of the dye molecule and an element in the cell, known as the chemo-receptor. This explanation is supposed to apply to all vital dyes. 2) The physical or phagocyte, theory, of Evans and Schulemann ('14, '15), that the colloidal dyes, such as trypan blue and lithium carmine, are taken into the cells by a process corresponding to phagocytosis and are housed in the form of chemically unchanged dye granules in the cytoplasmic vacuoles of certain types of cells. These authors do not include the more highly diffusible lipoid-soluble dyes, such as neutral red, Bismarck brown, and methylen blue, in the category of vital dyes which are phagocytized in this manner.

That the lipoid-soluble dyes stain only the preformed cell granules is the claim of most investigators. The affinity of methylen blue for the Nissl bodies of the nerve cells and of neutral red for the granules of mast cells are well-known examples of this kind of vital staining. That different granules are revealed by the use of different vital stains is also evident. The mitochondria, which stain specifically with Janus green, differ from certain larger granules which stain with neutral red, in certain eggs and in tissue cultures (M. R. Lewis, '17). Many authors have demonstrated two sets of granules in the same cells by a double stain of neutral red and methylen blue. However, it is apparent that even the highly diffusible dyes cannot be classified rigidly as stains which are deposited in the preformed cell structures only. Thus Arnold ('00) and Fischel ('01), both ardent advocates of the theory that vital dyes stain only preformed granules, give descriptions of the brilliant staining of the mucin content of the Leydig cells with neutral red. Fischel speaks of certain patches of neutral red in the gills of salamanders which he considers to be unchanged chemically, and Arnold's description of neutral red granules of various shapes, some of them

sharp-cornered, which occur in clumps or masses in certain cells, tallies exactly with the description of the dye concretions which accumulate in the pyrrhol cells. It might also be mentioned that the exact nature of these 'preformed granules' has not yet been discovered and, in this connection, Plato's ('00) observations are of interest. He studied the behavior of the cell vacuoles in living vorticella and found that it was the contents of these vacuoles which stained with neutral red. He also found that bacteria, spermatozoa, and red blood corpuscles, inside of leucocytes, were resolved into minute granules which stained with neutral red and which were indistinguishable from preformed granules. Stole ('02), in studying the effects of vital staining on amoebae, found that the contents of the vacuoles were the granules which stained with neutral red.

Thus it is possible that the more highly diffusible dyes may stain cell contents or secretions or may be stored as unchanged deposits of the dye, in addition to staining the preformed cell structures. On the other hand, there is evidence that the higher colloidal dyes may occasionally stain the preformed cell elements. M. R. Lewis ('17) notes that the cell granules which stain with neutral red, and which can be seen in unstained tissue cultures, also stain with pyrrhol blue. Kiyono ('14) mentions that the epithelial cells of the kidney, adrenal, and hypophysis, which are not otherwise phagocytic, take up the benzidine dyes. Evans and Schulemann ('15), while admiting that the cells referred to by Kiyono are stained by trypan blue and lithium carmine, claim that the type of staining in these cells, which is characterized by round droplets with regular outlines, is totally different from the true phagocytic staining of the macrophages where the dye is present as clumps of dark blue granules.

In the present observations, there appears to be evidence for both varieties of staining reaction on the part of the two highly diffusible dyes employed and also of the colloidal dye. And there is a conspicuous difference between those cells which show only a few round granules after staining (such as the epidermal cells with neutral red and Bismarck brown and the endothelial cells of the blood vessels with all three dyes) and those cells which take

up the dyes in large quantities. In the first type of cell, the granules do not increase in size or number with an increase in the intensity of the stain, while in the lymphatic endothelium, wandering cells, and mesenchyme cells the granules increase in number and size after an intense or prolonged staining with any of the three dyes. Since the work of Evans and Schulemann renders it highly probable that intense granular staining with trypan blue is evidence of a form of phagocytosis on the part of cells displaying this reaction, it seems natural to assume that an identical response of the same cells toward neutral red and Bismarck brown may also receive the same explanation.

That the lymphatics of the tadpole's tail possess a marked phagocytic power was shown by one of the authors (E. R. Clark, '09) in observations of the manner in which lymphatic sprouts grew toward red blood cells, extruded into the tissues, sent out processes toward them, and actively took them in. A recent observation, made in connection with injections of suspensions of carbon and carmine granules into the subcutaneous tissue of the tadpole's tail, is also of interest here. On certain occasions, when the suspensions were injected directly into the lumen of a lymphatic capillary, we noted that the granules of carbon or carmine soon made their appearance in the perinuclear areas of the lymphatic wall—the same regions which are filled with dye granules after vital staining. Wislocki's ('16) observation that lymphatic endothelium shows a strong avidity for trypan blue led him to formulate the hypothesis that the phagocytic lymphatic of Amphibia represents an intermediate stage in the evolution of the pyrrhol cell, since only specialized portions of the circulatory endothelium of adult mammals retain this property, and that, in early stages of development, the whole vascular endothelium might possess this power of phagocytosis.

The mononuclear wandering cells of the tadpole's tail, which stored the granules of trypan blue and neutral red to such a noticeable extent, undoubtedly belong to that class of cells which have been shown by various authors to react to vital stains of the benzidine group—the pyrrhol cells of Goldmann ('09), the macrophages of Evans ('15) and the histiocytes of Kiyono ('14).

These same cells have been shown by Ribbert ('04), Schleet ('07), Pari ('10), and Aschoff and Kiyono ('13) to take up lithium carmine in a similar manner. They correspond to the descriptions given by Ponfick ('69), Hoffmann and Langerhans ('69), and Siebel ('86) of those cells which take up particles of cinnabar. They are also the same cells which possess the power of storing fat, according to Anitschkow ('14), and which Kiyono ('14) has described as taking up colloidal silver. Metchnikoff ('92, '05) divided the mobile phagocytes, without much regard for their origin or morphological differences, into: 1) the macrophages, which react chiefly toward particulate matter and cell débris, and 2) the microphages, including the polymorphonuclear leucocytes, which show an especial avidity toward bacteria. The large wandering cells, which store vital dyes, have recently been called macrophages by Evans ('15) who aligns them with the macrophages of Metchnikoff as phagocytes of foreign particles. They have been assumed to be of tissue and endothelial origin (Aschoff and Kiyono, and Evans) and only rarely to occur in the blood stream.

That the division of the mobile phagocytes into two classes, made by Metchnikoff, is not a rigid one in all cases has been demonstrated by a number of observations. Rosenthal ('14) found, after injecting living non-virulent cocci into the blood stream, that the Kupffer cells of the liver and the reticulo-endothelial cells of the spleen (cells classified as macrophages) took up the organisms more rapidly than did the polymorphonuclear leucocytes. Similarly, Bartlett and Ozaki ('17) have shown that after injections of micrococcus aureus into the blood stream, 75 per cent of the organisms present in the liver and spleen were contained in wandering cells and in endothelial cells and only 25 per cent in the polymorphonuclear leucocytes, although in the lungs these percentages were reversed. And F. A. Evans ('14) found that while the polymorphs failed to stain after injections of filtered solutions of lithium carmine, they took up the dye particles after injections of unfiltered solutions, the mononuclear macrophages staining in both cases. Moreover, Downey ('17) has shown that the polymorphonuclear leucocytes will stain in a

typical manner wth trypan blue when they are outside the blood vessels or within a vessel which has been isolated from the circulation. The experiments with vital stains and injected fat, reported here, appeared to show that some of the leucocytes which were unstained while circulating became filled with dye granules after leaving the vessels. However, the observations also showed that not all of the wandering cells nor all of the migrated leucocytes of the tadpole's tail would store the granules of these vital dyes.

The present observations place the stellate connective-tissue cells of the tadpole's tail in the same category with the lymphatic endothelium and certain of the large wandering cells with regard to their mode of response toward these three vital dyes, although in no case was the accumulation of granules so great as in these two latter cell types. Most writers state that only the clasmatocytes or resting wandering cells of the connective tissue stain with the colloidal dyes. However, Evans and Schulemann ('14) mention briefly the finding of small blue granules in connective-tissue cells of the fibroblast type after staining with trypan blue. Moreover, Kiyono ('14) found a few silver spots deposited in the fibroblasts after injections of collargol. That the mesenchyme cells possess a definite power of phagocytizing particles of carbon and carmine injected into the tadpole's tail has been shown by the authors (see article Anat. Rec. Oct. 1918).

It is interesting to note that the three types of cell which display this especial avidity for storing vital dyes are identical with those which possess the power of ingesting particles of carbon and carmine. The similarity of the picture found in these experiments and in those recorded in the preceding article offers a strong argument for the phagocytic theory of vital staining in the case of these cells.

SUMMARY

Neutral red, Bismarck brown and trypan blue are true vital stains which can be used to advantage in the study of the living cells and tissues of the transparent tails of Amphibian larvae.

The more highly diffusible dyes, neutral red and Bismarck brown, stain the cells much more rapidly than the colloidal dye, trypan blue. But trypan blue has the advantage that it can be preserved in permanent preparations.

Neutral red and Bismarck brown stain small, regular, and apparently preformed granules in the cells of the epidermis. In addition, the contents of a richly branching subepidermal system are brilliantly stained with neutral red. Trypan blue does not stain any of the cells of the epidermis.

All three dyes stain an occasional and probably preformed granule in the walls of certain blood vessels.

Neutral red, Bismarck brown, and trypan blue are all deposited in a similar manner as large accumulations of dye granules in the perinuclear areas of the lymphatics, in certain large mononuclear wandering cells and leucocytes and, to a less degree, on the processes of the stellate connective-tissue cells. The physiological relationship shown by this reaction toward these vital dyes on the part of lymphatics, wandering cells, and mesenchyme cells is probably due to their common property of phagocytosis.

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Resumido por el autor, Edward Phelps Allis, Jr.

Sobre el origen de la hiomandíbula de los Teleostomos.

En Polypterus y otros Ganoideos las dos filas de branquias de cada uno de los arcos branquiales anteriores están reforzadas por radios branquiales cartilaginosos, y en Polypterus, las bases de dichos radios se han fusionado para formar una barra branquio-radial. Estas barras branquio-radiales se proyectan dorso-antero-mesialmente en cada arco formando un ángulo considerable con el epibranquial y faringobranquial del mismo arco y están dirigidas hacia puntos situados dorsalmente a la vena yugular. En los Selacios, una de estas barras ha dado lugar a los extrabranquiales de cada arco. En los Teleostomos barras semejantes han dado lugar, en el arco hial, a las cabezas articulares anterior y posterior de la hiomandibula, y en el arco mandibular a los procesos ascendente y ótico del palatocuadrado. El simpléctico es, probablemente, una parte de la barra branquio-radial anterior del arco hial y el interhial los elementos epales y faringeos del arco, fusionados y relativamente muy reducidos.

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AUTHOR'S ABSTRACT OF THIS PAPER
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ON THE ORIGIN OF THE HYOMANDIBULA OF THE TELEOSTOMI

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ONE FIGURE

In a work published in 1914, I came to the conclusion that there must be, in fishes, "a primarily somewhat independent mass of mesoderm cells lying lateral to the neurocranium and dorsal to the dorsal ends of the mandibular and premandibular arches, in the position of the pharyngeal elements of the branchial arches, which pharyngeal elements are wanting, as independent structures, in the mandibular and premandibular arches of all fishes." These cells were assumed to be capable of chondrification and to have given rise both to the ascending and otic processes of the palatoquadrate of the Dipneusti, Amphibia and Reptilia, and to the lateral wall of the trigemino-facialis chamber of fishes and mammals. Similar cells related to the hyal arch were said to have possibly given rise to some portion of the otic capsule, and its derivative the operculum, and probably also to the teleostean hyomandibula.

In a later work, published in 1915, I came to the conclusion that the cartilages derived from the mesoderm cells above referred to had, in all probability, their serial homologues in the extrabranchials and interarcual cartilages of the branchial arches of the Selachii, the posterior articular head of the teleostean hyomandibula being derived from the dorsal extrabranchial of the hyal arch and the anterior articular head from the interarcula cartilage between that arch and the mandibular arch. The symplectic was said to probably be a primarily independent cartilage, and probably an hypertrophied middle one or ones of the branchial rays of the mandibular arch. The single articular

head of the hyomandibula of the Chondrostei was said to apparently correspond to the anterior articular head of the teleostean hyomandibula. The hyomandibula of Polypterus was left largely out of consideration, but it was said that the suprapharyngobranchials of van Wijhe's ('82) descriptions of that fish and certain others of the Ganoidei were quite certainly represented in the extrabranchials of the Selachii, the suprapharyngobranchials accordingly being serial homologues of the posterior articular head of the hyomandibula.

Since the publication of the work last above referred to, I have had occasion to examine the branchial arches in Polypterus, and I not only find that the suprapharyngobranchials of van Wijhe's descriptions are simply the epibranchials of their respective arches, but that there are, in each arch, cartilages that quite certainly represent the special mesoderm cells that were assumed, in the two works above referred to, to have given rise, in the hyal arch, to the hyomandibula, and in the mandibular arch to the ascending and otic processes of the palatoquadrate.

Van Wijhe, in the work above referred to, described, in the dorsal half of the first branchial arch of Polypterus, a small cartilage that he considered to represent the epibranchial of the arch, and two bones that he called the supra- and infrapharyngobranchials, the infrapharyngobranchial apparently being considered by him to correspond to the typical selachian pharyngobranchial, and the suprapharyngobranchial to be a fifth element of a complete and typical branchial arch. The epibranchial is said to be almost completely concealed in a ligament that envelops both it and the infrapharyngobranchial, and that has its insertion on what Van Wijhe considered to be a part of the proötic covered by the thin lateral edge of the parasphenoid. The suprapharyngobranchial is said to be relatively large, to abut against a cartilaginous portion of the lateral wall of the neurocranium, and to have its distal portion deeply grooved to lodge the efferent artery of the arch. In the second and third branchial arches there is said to be no epibranchial, and it is said that the upper ends of the supra- and infrapharyngobranchials of those arches may be fused to form a short tube which encloses the efferent

artery of the arch. In the corresponding part of the fourth arch there is said to be a small pharyngobranchial, but in the figure given it is index-lettered as an infrapharyngobranchial.

In a 75-mm. specimen of Polypterus senegalus that I have examined in serial transverse sections, there is no trace of the independent so-called epibranchial cartilage described by van Wijhe in the first branchial arch, and I also find no trace of it in adult specimens of Polypterus bichir and Polypterus ornatipinnis. In the first branchial arch of the 75-mm. specimen, the supra- and infrapharyngobranchials of van Wijhe's descriptions are found as independent cartilages, and they are certainly simply, respectively, the normal epibranchial and pharyngobranchial of the arch. The epibranchial articulates by its distal end with the ceratobranchial of its arch, and by the anterior corner of its proximal end with the pharyngobranchial, and the posterior corner of its proximal end has been prolonged to form a stout process which has acquired articular relations with the lateral wall of the bulla acustica, there lying ventral to the vena jugularis and the truncus facialis. The pharyngobranchial articulates with the epibranchial, as above described, and, running anteromesially and somewhat ventrally, enters the angle between the lateral and ventral (horizontal) plates of the ascending process of the parasphenoid, and there has its attachment. In its course it lies imbedded in the lateral surface of the thymus, dorsal to a stout ligament that extends from the angle of the ascending process of the parasphenoid to the dorsal end of the ceratobranchial of the first branchial arch, this ligament being the one that is said by van Wijhe to envelop his epibranchial and infrapharyngobranchial. A groove on the external surface of the epibranchial (suprapharyngobranchial of van Wijhe), between it and the pharyngobranchial (infrapharyngobranchial of van Wijhe), lodges the efferent artery of the arch.

In the adult specimens of both Polypterus bichir and Polypterus ornatipinnis, I find strictly similar conditions; but the epibranchial and pharyngobranchial have apparently fused with each other, and each has undergone extensive ossification, the two so-formed bones being in contact at their distal ends and

there immovably connected with each other. The process on the posterior corner of the proximal end of the epibranchial has been completely ossified, excepting the articular cap by which it articulates with the lateral wall of the bulla acustica, and this process of the bone is strictly similar to that shown by van Wijhe in his figures of Amia and Lepidosteus, and by me in my figures of Amia and Scomber (Allis, '97, '03). In Amia I did not find the suprapharyngobranchial described by van Wijhe in that fish, but in Scomber, in a corresponding position, I found an independent piece of cartilage that articulated with the pharyngobranchial of the second branchial arch and that I called a suprapharyngobranchial.

In the second and third branchial arches of all my specimens of Polypterus, the 75-mm. one as well as the adults, the epibranchial and pharyngobranchial of each arch are completely fused with each other, and the anterior (lateral) and posterior (mesial) edges of the so-formed piece have been produced dorsally so that they touch, or fuse with each other, dorsal to the efferent artery of the arch, thus enclosing it in a short tube. The nerve of the arch runs posteriorly dorsal to this tube, not traversing it.

Each of the first three branchial arches of this fish is furnished with two rows of branchiae, each row supported by cartilaginous branchial rays the bases of which have fused to form a practically continuous bar of cartilage, as shown in the accompanying figure. The rays thus form a comb-shaped structure the base of which is arched in a curve that corresponds approximately to that of the branchial bar of the arch when the mouth is opened and the branchial chamber expanded. When the mouth is closed and the branchial chamber contracted, the levator muscles have pulled the distal (ventral) end of the epibranchial upward, and that element and the pharyngobranchial are then directed ventro-antero-mesially at a marked angle to the ceratobranchial. The comb-shaped structures formed by the branchial rays of each arch can not undergo a corresponding change of form, because of the relative rigidity of their basal bars, and the dorsal portions of those bars, the parts related to the epibranch-

ial and pharyngobranchial of each arch, project dorso-antero-mesially, but more mesially, and less anteriorly, than the dorsal portions of the branchial bar, this leaving a wide space between themselves and that portion of the branchial bar. The basal bars of the rays, projecting dorsally, lie external to the levator muscles of the arch, and their dorsal ends, which lie dorsal to the vena jugularis, are there attached by connective tissues.

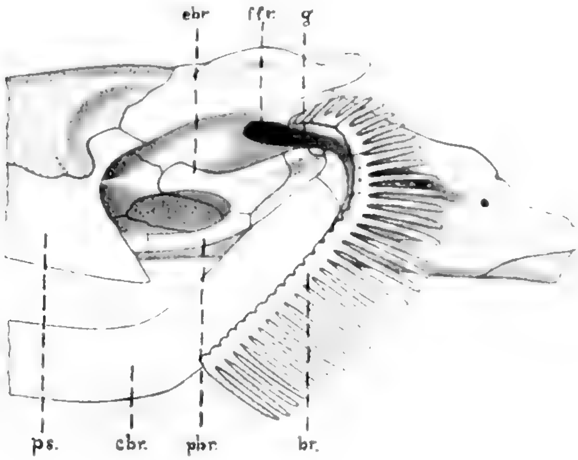

Fig. 1 Lateral view of the posterior portion of the neurocranium of Polypterus, showing the first branchial arch in place, with the related anterior series of cartilaginous branchial rays, but the posterior branchial arches and the hyal arch removed. × 2. *br.*, branchial rays of first branchial arch; *cbr.*, ceratobranchial; *ebr.*, epibranchial; *ffr.*, facialis foramen; *g.*, groove for vena jugularis; *pbr.*, pharyngobranchial; *ps.*, parasphenoid.

The nerve and efferent artery of the arch now pass ventral to the anterior one of these two branchial-ray bars, and then onward between the two bars onto the external surface of the ceratobranchial of the arch.

In Amia, Lepidosteus and Polyodon cartilaginous branchial rays similar to those of Polypterus are found, and their bases are in contact with each other, but not so completely fused as in Polypterus; and in these fishes, also, the branchial-ray bars project dorsal to the epibranchial and pharyngobranchial of the arch

to which they are related. There is accordingly every reason to believe that similar conditions existed in the immediate ancestors of these fishes, and that in those fishes branchial rays, capable of fusing with each other at their bases, were found also in the hyal and mandibular arches. The dorsal ends of the branchial-ray bars of the latter arches would then lie close to the bulging auditory portion of the neurocranium, dorsal to the vena jugularis, and hence in a position to form, in the hyal arch, a hyomandibula with one or two articular heads, and in the mandibular arch the ascending and otic processes of the palatoquadrate. The hyomandibula, thus formed, would lie in a plane somewhat inclined to that of the branchial bar of its arch, and the articulation of that bar with the hyomandibula would naturally be with its postero-internal surface, as is actually the case in Polypterus.

The hyomandibula of Polypterus is always said to have but a single articular head, and the nervus hyoideus facialis runs outward posterior to that head, and the nervus mandibularis facialis anterior to it. I however find, in my 75-mm. specimen of this fish, a small and independent bit of cartilage lying immediately posterior to the cartilage that caps the actual articular head of the hyomandibula, and the so-called accessory hyomandibula is developed in relation to it. On one side of one adult specimen that was also examined, I find the dorsal edge of the accessory hyomandibula capped with cartilage, that cap forming a posterior extension of the cap on the articular head of the hyomandibula; and Traquair apparently shows similar conditions in his figure of this fish ('70, fig. 6, pl. 6). On the other side of the head of my specimen, this edge of the accessory hyomandibula is covered with bone, this being as van Wijhe ('82) found it in the specimen described and figured by him. A stout ligament always extends from the head of the accessory hyomandibula to the dorsal edge of the opercular process of the hyomandibula, and lies posterior to the nervus hyoideus facialis; this ligament and the accessory hyomandibula thus quite certainly representing the posterior articular head of the teleostean hyomandibula. The efferent artery of the arch lies postero-internal to

this ligament, thus corresponding, in its relations to the branchial rays, to the posterior efferent artery of the Selachii. The position of the nervus mandibularis facialis, anterior to the anterior articular head of the hyomandibula, is doubtless due to this nerve having separated from the nervus hyoideus shortly after the truncus facialis issued from its foramen, thus permitting it to slip over the dorsal end of the anterior branchial-ray bar before that bar had acquired articulation with the cranial wall. The ventral end of the hyomandibula presents two angles, or processes, one of which articulates with the interhyal and is apparently formed by the posterior branchial-ray bar of the arch, while the other articulates with the quadrate, forms the so-called symplectic process of the hyomandibula, and is doubtless formed by the anterior branchial-ray bar. The interhyal must then represent the coalesced and relatively greatly reduced epal and pharyngeal elements of the arch.

In the Holostei and Teleostei the conditions are similar to those in Polypterus excepting in that the posterior articular head of the hyomandibula is more fully developed, and in that the nervus mandibularis facialis does not separate from the nervus hyoideus until after the truncus facialis has passed between the two heads of the hyomandibula.

In the Chondrostei, the posterior articular head of the hyomandibula is wholly wanting, this indicating that the posterior branchial-ray bar has more or less completely aborted, and, doubtless in correlation with this, the interhyal has acquired articulation with the symplectic which must be either a detached portion of the anterior branchial-ray bar or be derived, as suggested in my earlier work, from branchial rays of the mandibular arch.

In the Plagiostomi, the hyomandibula articulates with the cranial wall ventral to the vena jugularis, and is formed, in the Selachii, by the epihyal, and in the Batoidei by the pharyngohyal (Allis, '15). There is in these fishes but a single row of branchial rays, a posterior one, and it is found in the hyal as well as in the branchial arches. Associated with these rays there are so-called dorsal and ventral extrabranchials, which are currently considered to be simply modified dorsal and ventral ones of the

branchial rays actually found in these fishes. Braus ('06), however, considers them to belong to an independent category of skeletal elements, for, in embryos of Heptanchus, he found them lying not only at a considerable distance from the branchial rays, but also at right angles to those rays and parallel to the inner branchial bars. These relations to the branchial rays at once suggest a branchial-ray bar that has been developed either in relation to those dorsal and ventral ones of the posterior row that were primarily related to the pharyngeal and hyal elements of the arch. If the extrabranchials have this latter origin, which seems probable, then the dorsal one would be of similar origin to that here ascribed to the posterior articular head of the teleostean hyomandibula. The conclusions arrived at in my earlier works, and briefly stated in the opening paragraphs of the present article, would then have to be modified simply by the substitution of the "anterior branchial-ray bar of the hyal arch" in place of "an interarcual cartilage that lay between that arch and the mandibular arch," and the symplectic would be derived from the anterior branchial-ray bar instead of from the branchial rays of the mandibular arch.

The conditions here found in recent Teleostomi and Plagiostomi could evidently not be derived the one from the other without reversion to a type from which they both must have descended, and as there are no indications of any such reversion having taken place, the separation of the two lines here indicated must have taken place in very early geological times, for even in the crossopterygian Tristichopterus, remains of which are found in lower Devonian rocks, the hyomandibula must have articulated with the neurocranium dorsal to the vena jugularis, for Traquair ('75) says that the posterior margin of the palato-suspensory apparatus, "apparently corresponding to the hyomandibular element," gives articulation externally to the preopercular cheek-plate, and is itself connected dorsally with the squamosal region of the cranium; each of which conditions indicates that the hyomandibula lay external to the vena jugularis and articulated with the cranium dorsal to that vein.

Palais de Carnolès, Menton, France
April 13, 1918

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THE ANATOMICAL RECORD, VOL. 15, NO. 6
JANUARY, 1919

Resumido por el autor, Richard E. Scammon.

Sobre el desarrollo y fina estructura del cuerpo adiposo bucal.

En fetos de una longitud total de 6 a 8 cm. se encuentra generalmente un esbozo definido del corpus adiposum buccae. En fetos de 12 a 15 cm. de longitud dicho cuerpo ha adquirido próximamente su forma definitiva y lóbulos adiposos jóvenes sustituyen a las masas de tejido pre-adiposo existentes en estados anteriores. La formación de los lóbulos adiposos tiene lugar primero en la periferia del cuerpo del mismo nombre, particularmente en su parte anterior; desde esta región se extienden hacia dentro y hacia atrás, primero en su parte central y después en el tallo que le pone en relación con el corpus adiposus malae. La formación de nuevos lóbulos cesa generalmente al final del quinto mes de la vida fetal y el crecimiento ulterior del cuerpo se debe al aumento de tamaño de los lóbulos. Al principio este aumento se produce en parte por la formación de nuevas células adiposas en la periferia de los lóbulos y en parte también por el aumento de tamaño de las gotitas de grasa ya presentes en dichas células. La formación de nuevas células adiposas en el cuerpo adiposo bucal cesa generalmente al séptimo mes de la vida fetal y de aquí en adelante el crecimiento se efectúa generalmente por el aumento de tamaño de las células adiposas que se han formado previamente. La estructura microscópica del cuerpo adiposo del recién nacido es casi idéntica a la de la grasa general de la superficie, con la excepción de que los tabiques interlobulares son tal vez un poco más estrechos y están dispuestos algo radialmente con respecto al centro del cuerpo adiposo. Las secciones del cuerpo adiposo de adultos, obtenidas por congelación, presentan prácticamente la misma estructura que la mencionada en el niño.

Translation by Dr. José F. Nonidez,
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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ON THE DEVELOPMENT AND FINER STRUCTURE OF THE CORPUS ADIPOSUM BUCCAE[1]

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NINE FIGURES

The corpus adiposum buccae or sucking pad is a specialized and sharply circumscribed mass of adipose tissue which lies in the cheek partially wedged between the masseter and buccinator muscles and covered externally by the superficial fascia of the face and the zygomatic muscle. Posteriorly, it is connected by a stalk with a much larger fat mass, termed by Forster ('04) the corpus adiposum malae, which is located between the temporal and the pterygoid muscles and which possesses a superficial process extending over the outer surface of the temporal muscle beneath the temporal fascia.[2]

The sucking pad was apparently first mentioned by Heister in 1732, who, thinking it was glandular in character, termed it the glandula molares. Winslow, about twenty years later, again described the structure as a gland and wrote of a series of small

[1] This study was carried out with the aid of a grant from the Research Fund of the University of Minnesota.

[2] The body has received many names. Besides the term applied to it by Heister, under a misconception of its nature, the structure has also been called the boule graisseuse, boule de Bichat, Wangenfettpfropf, Wangenfettpolster, Saugpolster, sucking pad, and sucking cushion. It is not clear that the B. N. A. term, corpus adiposum buccae, which I have employed here, was originally intended for this particular fat mass; in fact, it is more probable that this expression was meant to indicate the entire mass of which the corpus adiposum malae forms the main body. However, most modern authors have used the B. N. A. term in the narrow sense of the sucking pad proper, and to avoid further synonymity I have followed their example. Berg ('11), in his classification of the fat masses of the body, places the corpus adiposum buccae in the category of intermuscular fat masses together with the adipose tissue between the layers of the temporal fascia and the orbital fat.

ducts which passed from it through the buccinator muscle to open into the oral cavity near the last molar tooth. Bichat recognized the true fatty nature of the sucking pad and referred to it in his Anatomie Générale in 1801. He is sometimes cited as the discoverer of the body. Bichat's remarks on the sucking pad are very brief and are purely incidental to a discussion on the presence of adipose tissue in early life. It is quite likely that the true nature of the body was known to anatomists before this time, although the examination of a large amount of the literature of the eighteenth century dealing with the anatomy of the fetus and child has failed to reveal any descriptions beyond those already mentioned.

The body as seen in the adult was figured by Burns in 1821, but it is not clear from this author's description that he regarded it as a normal structure. In 1852, Gehewe, in a Latin thesis, gave an excellent account of its gross anatomy and described its development in so far as it could be seen with the naked eye. Since this time the gross form and relations of the body have been figured and described by several authors, the most complete accounts being those of Ranke ('84), Lafite-Dupont ('00), Forster ('04), and Shattock ('09).

The phylogeny of the sucking pad has been studied in detail by Forster ('04). He finds that the entire mass of the corpus adiposum malae of the higher Primates is derived from the extra-orbital fat pad of the lemurs, which, in turn, is formed from an outgrowth of the periorbital fat mass of lower mammals. The corpus adiposum buccae, or facial extension of the corpus adiposum malae, is developed in the Primates as the orbital gland disappears and the muscles of mastication undergo partial regression.

Lehndorff ('07) investigated the chemical composition of the sucking pad and found it richer in the fats of high melting point (palmitic and stearic acids) and poorer in oleic acid than the general superficial fat. Shattock ('09), however, is of the opinion that the difference between the two is too slight to be of any great significance.

The function of the sucking pad has been discussed at length by Ranke ('84), Forster ('04), Lehndorff ('07), and Eisler ('12).

Very little has been written on the development and finer structure of the sucking pad. Gehewe ('52) found the first traces of the body in fetuses of the third month and noticed its gradual increase in size up to the time of birth. His studies were made entirely by macroscopic methods. Robin and Gimbert ('64) described the structure as appearing about the sixtieth day of fetal life as a number of clusters of small fat-cells. They found that the later growth of the body took place by the formation of new clusters as well as by the increase in the size of the earlier ones. Lafite-Dupont ('00) described the body in a fetus 12 cm. in length as consisting of a dense mass of mucous connective tissue, the fibers of which were arranged in the vertical plane of the face. This mass contained a few clusters of leucocytes. In a fetus of five months this body was transformed into a mass of adipose tissue and the embryonic mucous connective tissue had entirely disappeared. This transformation began in the central and lower part of the organ. Ranke ('84) figured and described the finer structure of the sucking pad in the late fetus and the new-born. He found it to consist of numerous lobules of unilocular fat-cells separated by broad septa of connective tissue. The whole body was surrounded by a definite capsule of fibrous connective tissue as well. A large number of blood-vessels ramified upon the outer surface of this capsule and their branches penetrated it to break up into terminal plexuses around the fat cells of the lobules. This description was confirmed by Shattock ('09), who also noted that the sucking pad was present in the fourth month of fetal life. Berg ('11) mentions that the body is in a fetus 10 cm. in length, although no fat-cells were observed at this stage.

DEVELOPMENT

The time of formation of the sucking pad, like that of most of the fat masses of the body, is subject to some variation, but the region which it will occupy later is clearly marked out in fetuses 4 or 5 cm. in total (crown-heel) length. At this time the lateral walls of the buccal cavity, which hitherto have been somewhat

compressed from side to side, commence to thicken considerably with the lateral extension of the developing maxillae, so that a broad band of tissue intervenes between the epithelium lining the oral cavity and the skin covering the cheek. The margins of this mass are already occupied by sheets of developing muscle—by the anlage of the buccinator medially, and by the facial portion of the sphincter colli laterally. These muscular sheets thus form the side walls of a region which is quadrilateral in frontal section and which is bounded by the maxilla above and by the mandible and the masseter muscle below. This region is closed anteriorly by the approach of the anterior part of the buccinator muscle and the oral portion of the sphincter colli, but posteriorly it becomes continuous with the pterygoid region and through it with the orbit which is as yet incompletely enclosed by its bony walls.

The region thus outlined may be termed for convenience the buccal space. It is filled with a delicate mesenchyma which is looser meshed than that of the face generally. In this mesenchyma are embedded the parotid duct and a coarse plexus of veins. The duct passes through the facial portion of the sphincter colli, crosses transversely through the buccal space, and, after piercing the buccinator muscle, opens into the oral cavity. The venous plexus arises from the large veins at the base of the orbit and passes obliquely downward through the space. It drains in part into the facial and in part into the internal maxillary vein. The radicles of this plexus, which are of extremely irregular caliber, are surrounded by a mass of loose-meshed mesenchyma, which, however, has not differentiated sufficiently as yet to be termed preadipose tissue. A frontal section of the cheek of a fetus of this stage is shown in figure 1.

A definitive anlage of the sucking pad is generally found in fetuses from 6 to 8 cm. in total length, although sometimes it does not appear until a little later. By this time the buccal space has become somewhat narrowed through the growth of the muscles of mastication, and the individual muscles which are formed from the facial portion of the sphincter colli are

clearly differentiated. The parotid duct pursues the same course through the space as it does in younger fetuses, but a definite connective-tissue sheath is now beginning to form around it. The molar glands are clearly differentiated, but

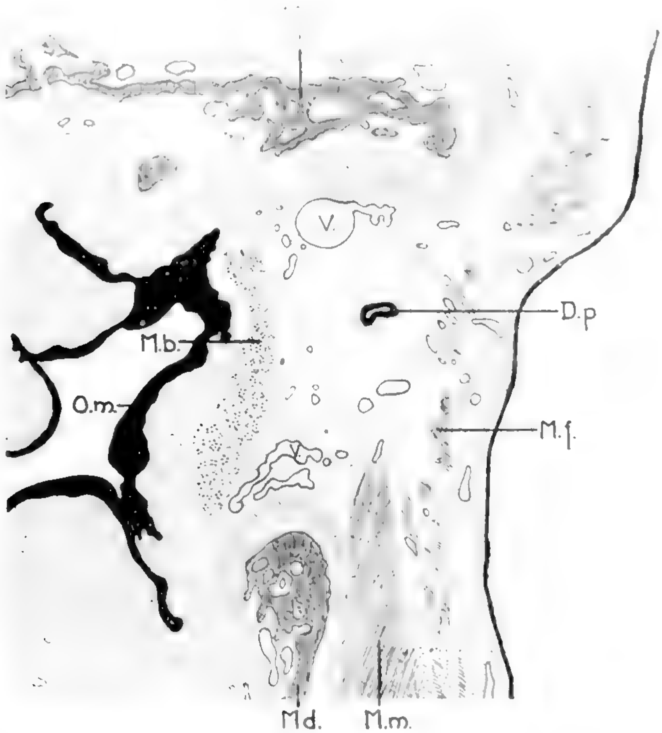

Fig. 1 Frontal section of the face of a human fetus, 60 mm. in total length, showing the region of the future sucking pad. Epithelial structures are represented in solid black, mesenchyma in stipple, muscle by short parallel lines or by coarse stipple, bone by close vertical ruling, and blood-vessels in solid outline. *D.p.*, parotid duct; *M.b.*, anlage of buccinator muscle; *Md.*, mandible; *M.f.*, anlagen of facial muscles; *M.m.*, anlage of masseter muscle; *Mx.*, maxilla; *O.m.*, oral epithelium; *V.*, venous plexus.

have not pierced the buccinator muscle. In two specimens of this stage which I have examined the orbital inclusion was located just lateral to the buccinator muscle and anterior to the internal pterygoid (fig. 2, *O.i.*).

By this time the arrangement of the veins in this region is considerably modified. The upper part of the plexus is differentiated into several trunks which connect with the inferior veins of the orbit above, while the lower part forms vessels which drain into the facial vein below. These lower trunks represent the vena ophthalmofacialis of Gurwitsch and Sesemann or the vena facialis profunda of French authors. The middle part of the original plexus connects posteriorly with the pterygoid plexus. It is broken up anteriorly into a number of small venules which anastomose freely. The sucking pad is in the process of formation around these venules. The periphery of the mass is slightly differentiated into a capsule which is indicated more by the direction of the fibers forming it than by a condensation of the tissue. Within this capsule the mesenchyma is wide meshed and delicate except immediately around the venules, where it is somewhat condensed, forming thin sheaths about the vessels. Mixed with the preadipose tissue are a considerable number of young blood-cells. These may be the result of an accidental extravasation from the smaller vessels into the tissue of the sucking pad, but I have observed them in three of the four specimens of this stage which I have examined, and apparently they were also seen by Lafite-Dupont in a somewhat older specimen. None of the epithelial structures which penetrate the buccal space lie in the immediate region of the anlage of the sucking pad at this time. Figure 2 is a drawing of a transverse section of the left cheek and neighboring structures of a fetus 7 cm. in total length and illustrates most of the important features of the sucking pad at this stage.

In fetuses from 12 to 16 cm. in total length the corpus adiposum buccae has approached its final form and young fat lobules are commencing to replace the preadipose tissue seen in earlier stages. The mass now fills the outer part of the buccal space. It is surrounded by a definite capsule of developing fibrous tissue. Within this capsule the organ consists of a meshwork of fibers of young connective tissue in which are embedded a few developing fat lobules and the plexus of veins already described. The fat lobules are confined almost entirely to the

periphery of the anterior part of the organ. They consist of preadipose tissue and true fat cells. The latter, which are quite small, are found mainly in the centers of the lobules. The duct of the parotid gland comes in contact with the capsule of the sucking pad, but does not penetrate it. The molar glands

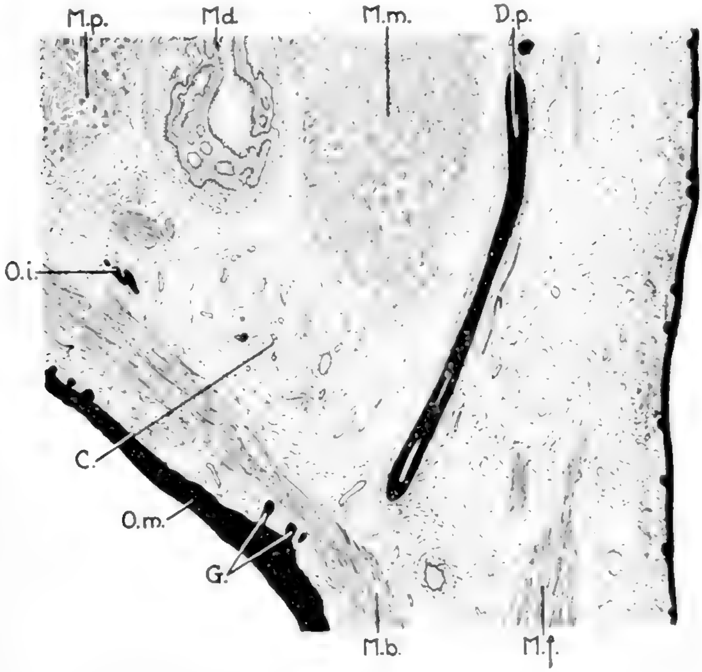

Fig. 2 Transverse section of the left cheek of a human fetus 7 cm. in total length. Method of drawing similar to that employed in figure 1. *C.*, corpus adiposum buccae; *D.p.*, parotid duct; *G.*, anlagen of molar glands; *M.b.*, buccinator muscle; *Md.*, ramus of mandible; *H.f.*, facial musculature and fascia; *M.m.*, masseter muscle; *M.p.*, internal pterygoid muscle; *O.i.*, orbital inclusion; *O.m.*, oral mucous membrane.

are now embedded in the substance of the buccinator muscle, but they do not come in direct contact with the capsule of the sucking pad. Small fat lobules are in the process of formation, both external to the facial musculature and fascia and also in the portion of the buccal space which is not occupied by the sucking pad and by epithelial and vascular structures. The

condition of the sucking pad at this stage is illustrated by figure 3, a frontal section passing through the extreme anterior part of the body in a fetus 15 cm. in total length.

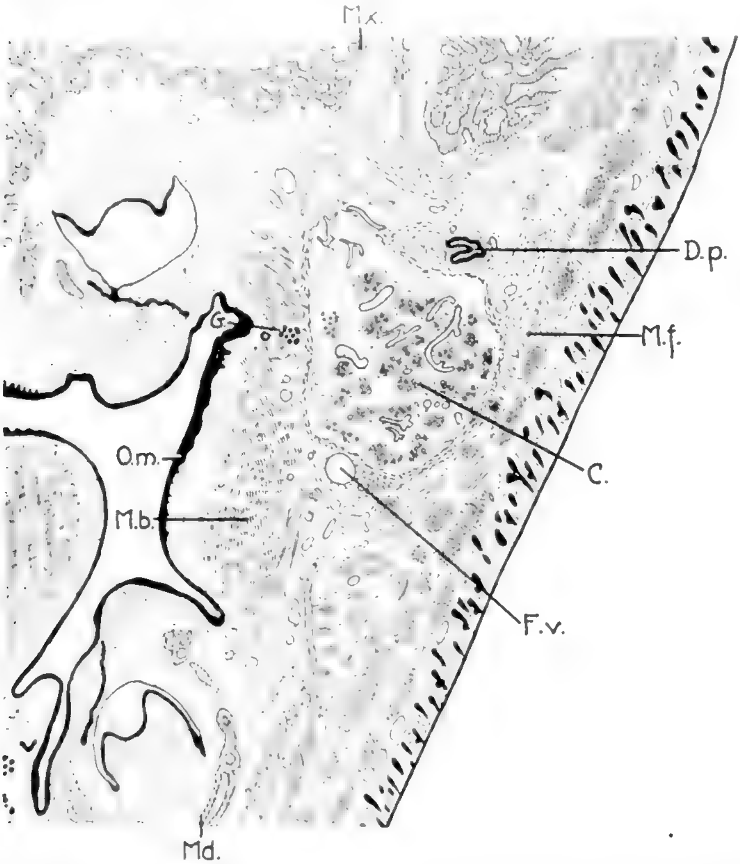

Fig. 3 Frontal section of the left cheek of a human fetus 15 cm. in total length. The section passes through the extreme anterior part of the sucking pad. *C.*, corpus adiposum buccae; *D.p.*, parotid duct; *F.v.*, facial vein; *G.*, molar glands; *M.b.*, buccinator muscle; *Md.*, mandible; *M.f.*, facial muscles and fascia; *Mx.*, maxilla; *O.m.*, oral mucous membrane.

After the sucking pad has reached the stage just described, it grows rather rapidly. It expands outward and also backward over the superficial surface of the masseter muscle and con-

tributes considerably to the rounded form of the cheek which is so noticeable in human fetuses of the latter half of intra-uterine life. In this expansion the capsule of the body is carried outward towards the facial muscles and fascia, and the broad band of mesenchymal tissue which formerly separated these structures is reduced to a narrow sheet which contains a rich plexus of veins and a few small fat lobules. The medial portion of the capsule is also pressed inward towards the buccinator muscle, but an intermediate strip of mesenchymal tissue, which contains the bodies of the molar glands, still persists in this position. As in earlier stages, the parotid duct and the molar glands lie entirely outside the capsule of the sucking pad. They now possess definite mesenchymal envelopes which are independent of it. These relations are shown in figure 4, a drawing of a transverse section of the right cheek of a fetus 17.5 cm. in total length.

The finer structure of the sucking pad during this period of rapid growth is somewhat variable. As was pointed out in the description of the preceding stage, the formation of fat lobules takes place first at the periphery of the body and particularly in its anterior part; from this region the process extends inward and backward first into the center of the body and then into the stalk which connects it with the corpus adiposum malae. The lobules are always formed around the first branches of the venous plexus. Figure 4 shows a stage at which the peripheral lobulation of the body is well under way, while the central portion of the mass contains almost no differentiated adipose tissue. Thus the early expansion of the sucking pad is not dependent upon the formation of fat lobules, but upon the growth of the mesenchymal meshwork in which they will appear later. As the lobules are developed the connective tissue between them is reduced to the form of broad septa. It seems probable that the formation of new lobules is completed, in the majority of cases at least, by the end of the fifth fetal month. I estimate that the body contains from 250 to 350 lobules at this time.

The blood supply of the sucking pad can best be studied at this period while the lobules are still separated by broad connective-tissue septa. The arterioles which supply the body enter

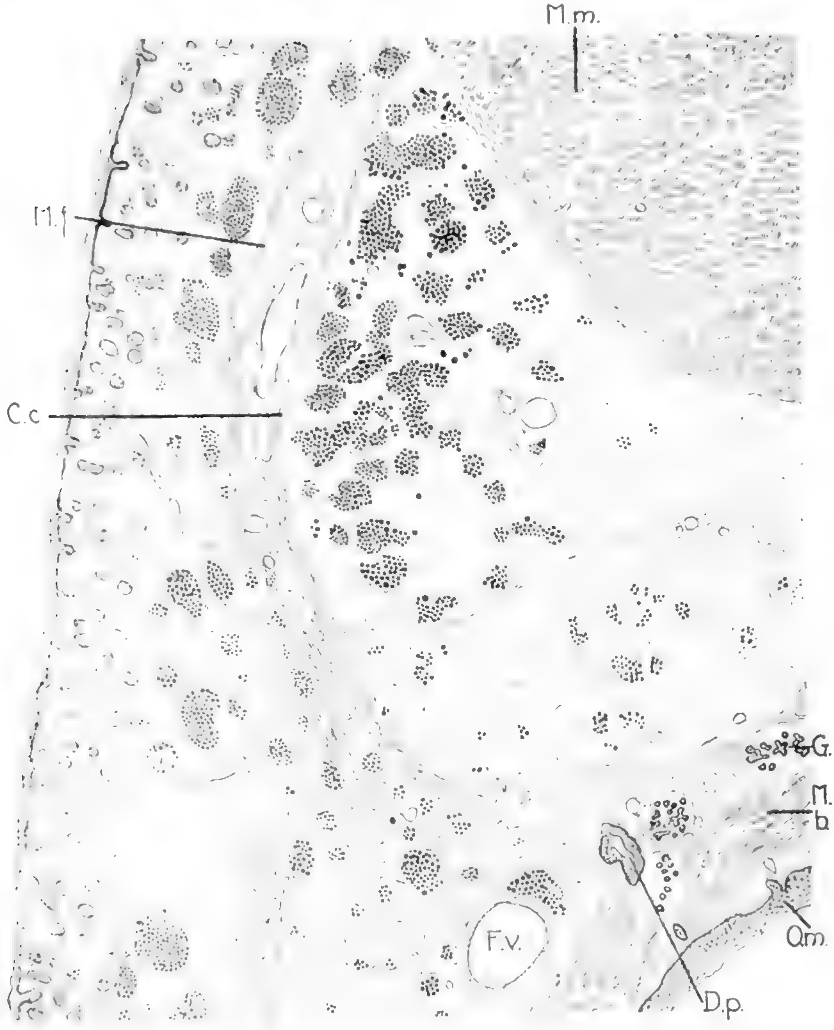

Fig. 4 Frontal section of the right cheek of a human fetus 17.5 cm. in total length. The specimen was stained with scarlet red and the colored fat droplets are represented in solid black in the drawing. *C.c.*, capsule of corpus adiposum buccae; *D.p.*, parotid duct; *F.v.*, facial vein; *G.*, molar glands; *M.b.*, buccinator muscle; *M.f.*, facial muscles and fascia; *M.m.*, masseter muscle; *O.m.*, oral mucous membrane.

its capsule from all directions, and end, after passing along the septa, by breaking up into capillary plexuses among the fat-cells of the lobules. The veins of the body are much more conspicuous than the arteries. They arise in the lobules and pass into the septa where they unite and finally form vessels of the third or fourth order. These vessels pass through the capsule and drain into the larger veins in the surrounding areolar tissue. Eventually most of the blood from the sucking pad is drained into the ophthalmofacial and facial veins. The vessels of the sucking pad are shown in figure 5— a frontal section of the body of an injected fetus 187 mm. in total length.

The subsequent changes in the body to the time of birth consist mainly in the expansion of the individual fat lobules and the reduction in thickness of the septa which separate them. With these changes the blood-vessels become much less prominent. The chronology of these later changes is subject to considerable variation, being apparently more dependent upon the nourishment of the fetus than upon its age. In some instances the fat lobules expand rapidly at an early period, so that at six months they are closely pressed against one another and are irregularly hexagonal or pentagonal in outline when seen in section. The connective-tissue septa in these cases are reduced to slender strands composed of flattened cells and fibers. In other cases this process may not take place until much later—sometimes not before the last month of fetal life. It is possible that the difficulty in suckling experienced by some premature and ill-developed infants may be due in part to the incomplete development of the sucking pad.

As has been stated, the formation of new fat lobules in the sucking pad generally ceases by the end of the fifth fetal month and the later growth of the body is due to the increase in the size of the lobules. At first this increase is brought about in part by the formation of new fat-cells at the periphery of the lobules and in part by the enlargement of the fat droplets already present. The formation of new fat-cells generally ceases in the seventh fetal month, and thereafter, as a rule, growth takes

place only by the enlargement of the fat-cells which are already formed.

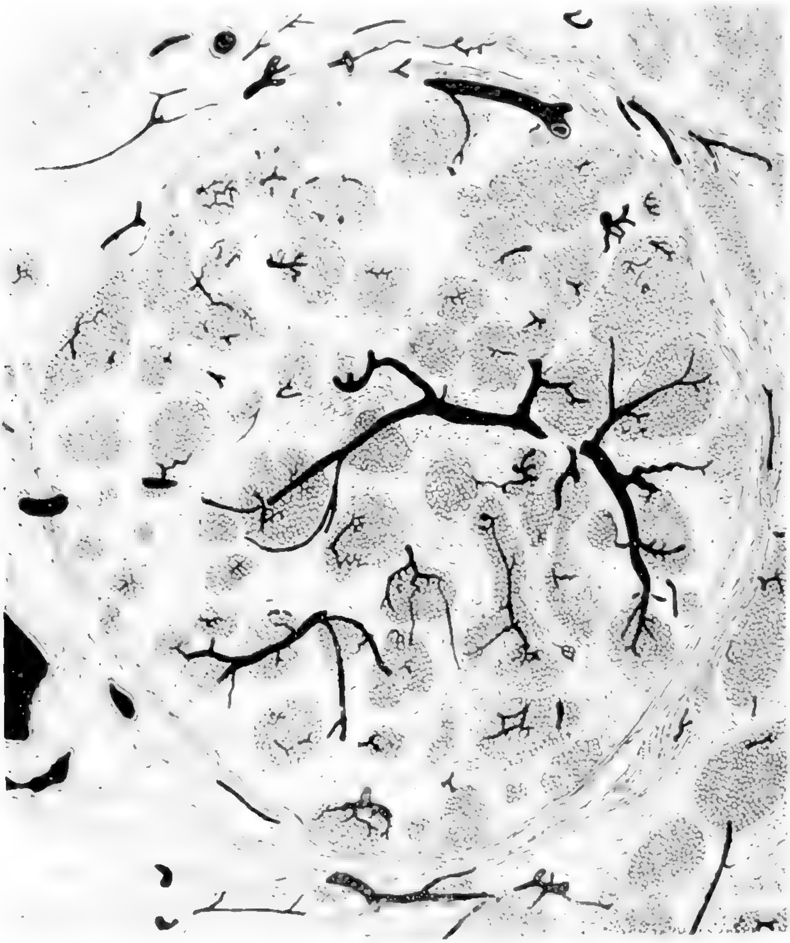

Fig. 5 A frontal section of the sucking pad of a human fetus 18.7 cm. in total length. The veins of the specimen have been injected and are represented in solid black in the drawing. The fat-cells are represented by small circles.

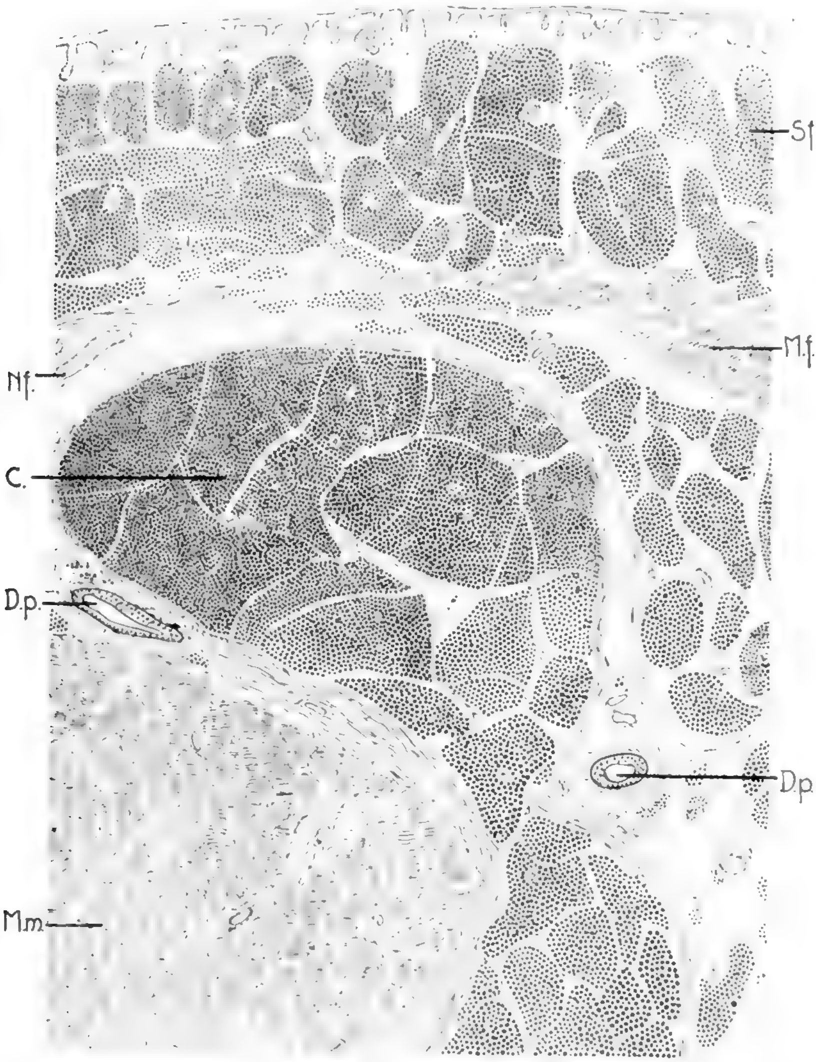

Fig. 6 A portion of a transverse section of the left cheek of a human fetus 32 cm. in total length. The section was stained with scarlet red and the colored fat droplets are represented in solid black in the drawing. *C.*, corpus adiposum buccae; *D.p.*, parotid duct; *M.f.*, facial musculatur and fascia (the leader enters the zygomatic muscle); *M.m.*, masseter muscle; *N.f.*, branch of facial nerve; *S.f.*, superficial fat lobules.

STRUCTURE OF THE SUCKING PAD AT BIRTH

At birth the sucking pad is a prominent structure of the cheek. In well-nourished individuals it is expanded to such a degree that it pushes the buccinator muscle inward towards the oral cavity and forms a prominent elevation laterally on the external surface of the face. The expansion of the body has forced its capsule outward against the superficial fascia of the face and inward against the fascia covering the buccinator muscle. Only a small cleft containing areolar tissue and blood-vessels separates the capsule of the body from these fascial planes. It is due to the presence of this space that the sucking pad is so easily dissected out in the new-born. This separation between the capsule and the investing fascia is so readily accomplished that the body was at one time described as partially surrounded by a bursa (Verneul, '57). Sections of this region, however, show no evidence of such a structure. Figure 7 is of a frontal section of the face of a very well-developed and nourished new-born child weighing 4050 grams. It shows the sucking pad in a high state of development.

The finer structure of the body at birth is almost identical with that of the general superficial fat except that the interlobular septa are perhaps a little narrower and are somewhat radially arranged in regard to the center of the body, while those of the superficial fat are placed at right angles to the surface of the skin (fig. 8).

POSTNATAL HISTORY

Comparatively little is known of the postnatal history of the corpus adiposum buccae. Gehewe ('52) stated that the body persists throughout life and that he had observed it in the emaciated cadaver of a woman over sixty years of age. Robin and Gimbert ('64), on the other hand, found little change in its size during the first four or five years of life, but concluded that after that time it diminished with age and with disease. Lafite-Dupont ('00) also thought that the body became smaller with age. Ballantyne ('91) agrees with Gehewe that the body per-

sists in maturity, as also does Shattock ('09). The survival of the structure during the wasting diseases of infancy is a common clinical observation and has been commented upon by Ranke ('84), Lehndorff ('07), and others. As has been stated, Allen Burns ('21) was apparently the first observer to figure the structure accurately in the adult. Modern treatises on adult

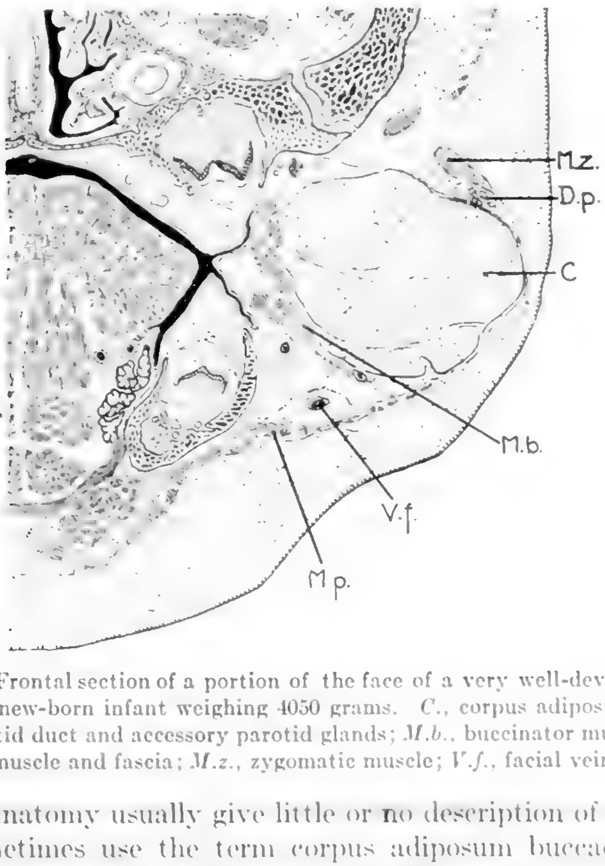

Fig. 7 Frontal section of a portion of the face of a very well-developed and nourished new-born infant weighing 4050 grams. *C.*, corpus adiposum buccae; *D.p.*, parotid duct and accessory parotid glands; *M.b.*, buccinator muscle; *M.p.*, platysma muscle and fascia; *M.z.*, zygomatic muscle; *V.f.*, facial vein. × 2½.

human anatomy usually give little or no description of the body and sometimes use the term corpus adiposum buccae for the general fat mass of the cheek and not for the sucking pad proper. However, the body is briefly described in connection with the mouth by Jonnesco in Poirier and Charpy's Traité d'Anatomie and in detail by Eisler in Bardeleben's Handbuch.

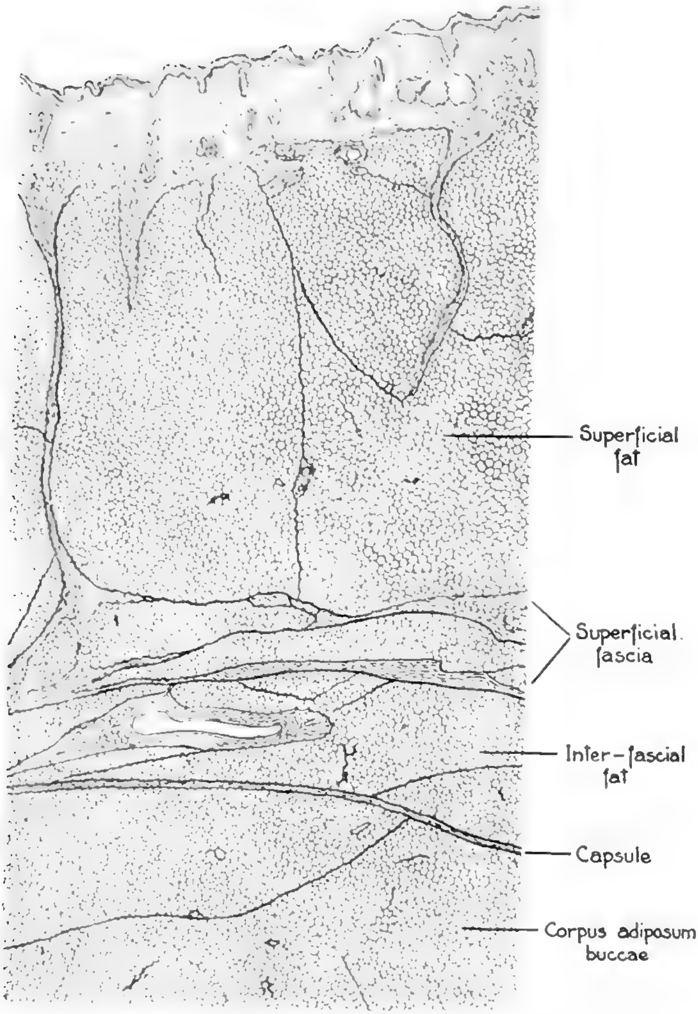

Fig. 8 A section passing through the skin, superficial fascia, and outer part of the sucking pad of a well-developed and nourished new-born infant weighing nearly 4000 grams.

In order to determine the usual condition of the sucking pad in the adult, a series of forty-two cadavers was examined in the dissecting room. The body was well developed in thirty-four of these cases and in two other instances it was present and well developed on one side of the face and practically absent on the other. This series of cases included the bodies of individuals from about twenty to about sixty years of age. So far as could be observed there was no relation between the size of the sucking pads and the age of the individual. A number of the cadavers of this series were of persons who had died in an advanced stage of tuberculosis; in some of these cases the superficial adipose tissue of the body was reduced to the minimum, but the sucking pads showed little or no reduction in size. It is evident that wasting disease, in the adult as in the suckling, has little effect upon the sucking pad.

The body in the adult may occupy the fossa bounded by the masseter, the buccinator, the zygomatic, and the platysma and risorius muscles, or it may extend forward and outward over the external surface of the masseter. The parotid duct, as in the fetus and the infant, may either pass cranial to the body or may lie in a deep groove on its superficial surface. Figure 9 shows several sketches of the body in adult cadavers. Figure 9, C, is of an individual who died in an advanced stage of phthisis.

Frozen sections of sucking pads of adults show practically the same structure as that seen in the infant.

PHYLOGENETIC SIGNIFICANCE

It has been suggested that the corpus adiposum buccae of the higher Primates represents the framework of the orbital gland which is so well developed in the Carnivora and of which the molar glands of man are a vestige. This view was first advanced, I think, by Lafite-Dupont ('00). In my opinion, neither the phylogenetic studies of Forster on the sucking pad nor my observations on the development of this structure support this hypothesis.

Forster finds that the sucking pad is a specialized portion of a fat mass which takes its origin from the extra-orbital fat body of the lemurs and which only secondarily enters the buccal region in the higher Primates. It is thus a body from another area

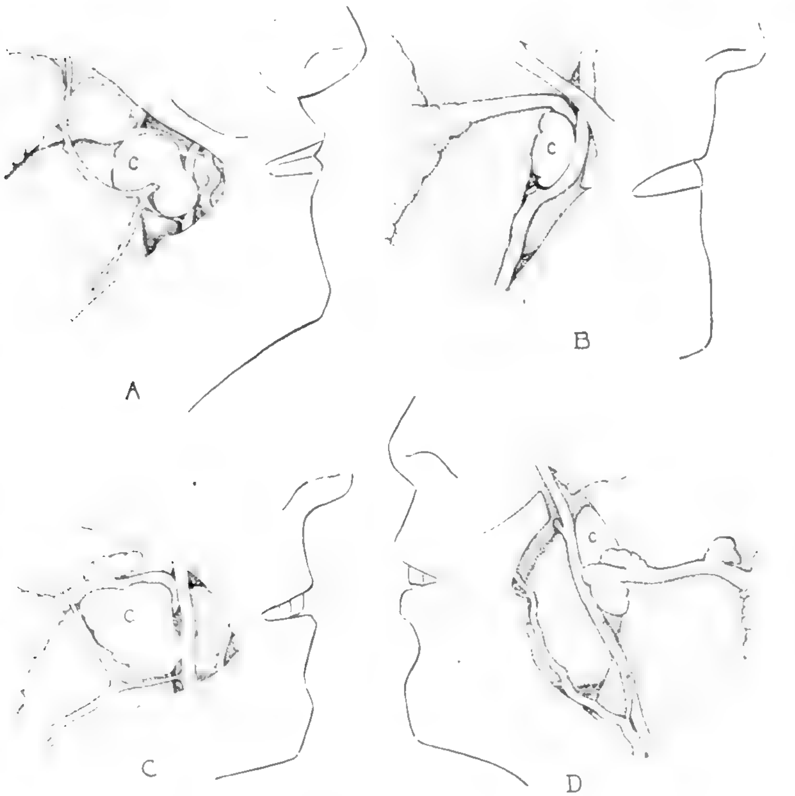

Fig. 9 Four sketches of dissections of the sucking pad in adult cadavers. C., corpus adiposum buccae.

which invades the region of the orbital gland and fills the space formerly occupied by that organ, but it is in no sense the remains of it.

The sucking pad, in its development, is built up around a venous plexus and not around any element of the orbitoparotid gland complex. The epithelial elements of this complex in man

(the parotid duct, the orbital inclusion, and the molar glands) do enter the area which I have termed the buccal space, but they do not enter the territory which is later to be incorporated in the sucking pad and they do not pierce the capsule of this structure after it is differentiated. In fact, the molar glands, which are considered to be the vestiges of the orbital gland, do not enter the buccal space at all until long after the sucking pad has been differentiated and a definite capsule has formed around it.

In Carmalt's paper ('13) on the anatomy of the adult salivary glands in man the statement is made that "The molar glands, when present, are for the most part embedded in the ento-masseteric fat mass of the 'sucking pad.'" While these glands lie in the loose adipose and areolar tissue of this region which fills the space between the corpus adiposum buccae and the muscles on either side of it, I have not observed them penetrating the capsule of the sucking pad proper, and the relation between them and the sucking pad is one of juxtaposition only. I think, therefore, that it may be safely concluded that while the sucking pad replaces the orbital gland in position in the higher Primates and in man, it is not to be regarded as a vestige of that structure.

SUMMARY

1. The corpus adiposum buccae is a sharply circumscribed mass of fat lobules which are formed around the radicles of the middle part of the venous plexus which connects the orbital veins with the superficial veins of the face. It is differentiated within a fairly well-marked area of the cheek which may be termed the buccal space.

2. The general region in which the sucking pad arises is mapped out in fetuses 4 or 5 cm. in total length and a definitely encapsulated area is well marked in fetuses 8 to 10 cm. in length. Fat-cells appear at this stage or a little later. They are arranged in lobules which are first found in the periphery of the anterior part of the body.

3. The body grows rapidly after the encapsulated area has been established. Most of this early growth is due to the expansion of the enclosed mesenchymal and preadipose tissue and not to the growth of fat-cells.

4. The later growth of the body is due to an increase in its fat content. This is brought about: *a*) by the increase in the number of fat lobules; *b*) by the formation of new fat-cells, and *c*) by the growth of the individual fat-cells. The formation of fat lobules generally ceases by the end of the fifth fetal month. The formation of new fat-cells ceases at a variable time in later fetal life, generally in the sixth or seventh fetal month, but sometimes not before the last fetal month.

5. The finer structure of the fully developed corpus adiposum buccae does not differ from that of ordinary superficial adipose tissue except that the interlobular septa are somewhat narrower and are arranged radially in the body.

6. The body persists in adult life in the large majority of cases. The presence of the sucking pad in later life is apparently not dependent on nutrition, as it may be found well developed on one side of the face and absent on the other in the same individual. It is also found well developed in individuals dead of wasting disease.

7. Observations on the development of the sucking pad in man offer no support to the theory that the body represents the remains of the orbital salivary gland. The sucking pad is developed quite independently of the parotid duct, the molar glands, and the orbital inclusion, and these structures never penetrate it. The molar glands do not approach the area in which the sucking pad is formed until some time after that structure is well established.

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Resumido por el autor, Frank Blair Hanson.

Orificios neurales en la escápula del cerdo. Una relación peculiar existente entre la rama dorsal de varios nervios espinales y la supraescápula del cerdo.

En los embriones y adultos del cerdo existe una supraescápula permanente, de gran tamaño. Las ramas dorsales o ramas posteriores primarias de los cuatro o cinco primeros nervios espinales pasan a través de los orificios neurales de la supraescápula no osificada. Estos orificios neurales, que nunca se han encontrado en otros mamíferos, existen constantemente en todas las especies de la familia Suidae, con la sola excepción de los pecaris. La presencia de estos nervios en el cartílago está producida por el desarrollo del precartílago, que crece sobre y alrededor de ellos antes de la aparición del cartílago. Los nervios mencionados no pasan nunca por fuera de la escápula en ningún periodo de la vida del cerdo.

Translation by Dr. José F. Nonidez,
Columbia University

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, DECEMBER 23

NERVE FORAMINA IN THE PIG SCAPULA

A PECULIAR RELATION EXISTING BETWEEN THE DORSALIS BRANCH OF SEVERAL SPINAL NERVES AND THE SUPRASCAPULA IN THE PIG

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TWENTY-ONE FIGURES

I. INTRODUCTION

Several years ago Prof. J. Sterling Kingsley ('00), in studying sagittal sections of a pig embryo, found the scapula in several sections to be completely segmented into five parts. Upon giving the matter closer attention, it was determined that four nerves passed through the cartilaginous suprascapula and that in this particular section the foramina of all four nerves had been cut through as shown in figure 1.

This fact was noted in a paper read before the American Morphological Society of New Haven, December 27, 1899. The following quotation from Professor Kingsley's paper is a condensed report of the main findings of that paper as published in Science, N. S., vol. 11, p. 167, and is, so far as I am aware, the only reference to be found in the literature bearing upon this matter.

In embryo pigs 18 to 60 mm. long, the dorsal crest of the scapula presents four foramina through which pass dorsal nerves, arising from the second to fifth thoracic ganglia, and passing directly to the skin. These were regarded as possibly indicating that the scapula was made up of metameric parts, and it was pointed out that these results were in full accord with the recent studies of Bolk upon the muscles of the shoulder girdle. They might be interpreted as adverse to Gegenbaur's views as to the origin of the girdles.

Upon the suggestion of Professor Kingsley, the author of this paper undertook to determine several points in regard to the history of these nerves. In the first place, which nerves are they; second, how do they get into the cartilage, and, third how do they get out again? It was also the author's purpose to discover if a similar condition be general throughout the mammalian series.

Concerning this last point all the evidence is negative. I have looked through series of serial sections, dissected embryos, or examined the skeletons of the opossum, mouse, rabbit, seal, bat, manatee, cat, sheep, and man. These forms represent most of the major groups of mammals, but in none is there any indication of the condition as found in the pig and described in the following pages. In his monograph on the shoulder-girdle, Parker ('68) pictures the scapulae of representatives of all the orders of mammals, many of them with a well-developed cartilaginous suprascapula. But in no case throughout the whole series is there any hint of the passage of nerves through this region. However, Parker does not give any figures of the pig scapula; his only reference to this form being three drawings of the pig sternum. Had the condition as in the pig obtained in other mammals he could hardly have failed to notice it, as in all the larger pig embryos the foramina are plainly visible to the naked eye.

Flower ('85) gives a figure of the scapula of the red deer with a large suprascapula, also descriptions of the scapula in the horse, hippopotamus, tapir, hyrax, and elephant among the Ungulates. He makes no mention of nerve foramina, yet could hardly have handled these bones without seeing them, had they been present.

Since the last two paragraphs were written the author has had the privilege of examining several hundred skeletons of mammals in the U. S. National Museum at Washington, D. C. It was observed that Ungulates in general are possessed of a suprascapula in the adult condition, sometimes but feebly developed. However, of all the skeletons reviewed in the museum, there was none outside the family Suidae with nerve foramina. Of

the genera of the Suidae the following were examined: Tayassu of Honduras, Sus barbatus of Borneo, Sus barbatus of West Borneo, Babirussa of Celebes, and the peccaries. The peccaries alone of all the genera above mentioned were lacking in respect to nerve foramina in the suprascapula. Tayassu, Babirussa, and Sus barbatus are so essentially like the domestic pig in this respect that a separate description is unnecessary. In view of the foregoing facts, this author is prepared to say with considerable emphasis that nerve foramina in the scapula are limited to the family Suidae, the peccaries alone excepted.

While these nerves were not found passing through the scapula in other mammals, even in so closely related forms as the sheep and the peccary, they were always present in the pig. More than fifty pigs have been examined, ranging in size from an embryo 18 mm. in length to an old hog weighing 450 pounds. Nerve exits through the suprascapula, varying in number from two to five, were found in every specimen studied. The author believes this to be a normal and constant condition in the pig, but apparently lacking in the other groups of mammals.

My thanks are due to Prof. J. Sterling Kingsley for the initial suggestion; to the authorities of the U. S. National Museum for supplying valuable material, and to Miss Bertha Uhlemeyer for assistance with the reconstructions and drawings.

II. MATERIAL AND METHODS

Pig embryos of 18 mm. in length to birth, scapulae from pigs of a few days after birth to young adult life, and one scapula from an over-sized hog of 450 pounds weight were used in this work. The smaller embryos were cut in transverse sections, camera-lucida drawings were made and the parts reconstructed in wax. For the larger embryos and postnatal specimens it was possible by gross dissection to trace the nerves directly from the spinal cord through the foramina in the suprascapula and to their distribution in the skin of the scapular region. Figure 3 is such a dissection, the specimen being approximately one week old. Figure 2 is a reconstruction in wax of the spinal cord,

spinal nerves, and the upper part of the scapula of an embryo 37 mm. in length.

III. IDENTIFICATION OF THE NERVES

In several of the series of sections the nerves could be traced from the foramina in the suprascapula back to the spinal cord. Figures 2 and 3 indicate clearly that the nerves are the dorsalis or primary posterior branches of spinal nerves.

Figure 2 is from a reconstruction in wax of a 37-mm. embryo indicating the structures under discussion. The two nerves, after passing through their scapular openings, divide and ramify over the skin of this region. From a study of the series of sections from which figure 2 was made and also from the gross dissection of nerves as shown in figure 3, it is demonstrated that these are the internal or cutaneous branches of spinal nerves, using the nomenclature of the human anatomists.

In determining which spinal nerves were affected, the method of human anatomy (Cunningham, '15) was adopted, i.e., of counting the first nerve behind the first true rib as the first thoracic spinal nerve. In the model from which figure 2 was drawn there are two nerves passing through the cartilage, and it was determined by the above-described method that these are the third and fourth thoracic spinal nerves. In the series of a 47-mm. pig (fig. 4) there are found three nerves going through the suprascapula at approximately the same level. These are the second, the third, and the fourth spinal nerves. In a 72-mm. embryo there were four nerves in the cartilage, the fourth probably being, though not positively identified, the first spinal nerve. At least in other specimens, figure 3 being an example, the first spinal nerve seems to take this course. Kingsley in the quotation given above says the nerves arise from the second to the fifth ganglia.

It should be noted here that in successive stages of growth there is an increasing number of nerves present in the cartilage of the scapula. Thus in 25-mm. and 37-mm. embryos there are two of these nerves, the third and fourth spinals as shown

in figures 2, 4, and 5; while in a 47-mm. pig there are three, and in the 72-mm. embryos four are present.

Figures 4 and 5 are drawings from wax models of 27-mm. and 47-mm. pigs, respectively, and show the position of the foramina in the future suprascapula. As these two figures are drawn to the same scale, they show well the interstitial growth carrying the nerves with it. Figure 5 is interesting as two nerves are firmly embedded in cartilage, while a third is just at the edge of the procartilaginous or growing end of the scapula. Figure 6 is a camera-lucida drawing from a transverse section of the 37-mm. stage showing the passage of one of the nerves through the cartilage of the shoulder-blade.

It should be stated, however, that the progressive increase in the number of nerves paralleling the growth of the embryo as noted above does not hold true for the series of nine young and adult scapulae represented by figures 13 to 21, inclusive. These show great variation in the number and position of the nerve openings, and will be described in some detail later on.

IV. ENTRANCE OF THE NERVES INTO THE SCAPULA

The question as to how these nerves came to be in the suprascapula is easily answered. It is a well-known fact that the nervous system is one of the earliest to be laid down in the embryo. In an 18-mm. pig the scapula is still in the procartilage stage, while the central and peripheral nervous system is a well-established structure. Figure 7 is a camera drawing of a transverse section through an 18-mm. stage of the pig. The nervous system is here seen to be in an advanced stage, while the patch of loose mesenchymatous material is the anlage of the future scapula. In this figure is also seen a spinal nerve in close proximity to the procartilaginous scapular anlage. Figures 8 and 9 are designed to show how the procartilage in its growth surrounds and invests the nerves which lie in its path.

In figure 8 there is shown a transverse section of a 25-mm. embryo in which one end of the scapula is clearly cartilaginous, while at the other end the procartilage may be seen forming

around a spinal nerve. As the scapula grows it gradually includes additional nerves, thus explaining why the different stages have a varying number of nerves, from one in the smallest embryo to five in the full-term fetus. Figure 9 is a camera drawing from a transverse section showing the scapula, and, lying just above it and bent around it in a V-shaped manner, one of the dorsalis branches of a spinal nerve. In a little later stage of development a patch of procartilage will appear dorsal to the nerve as in figure 8, and the gradual union of the two pieces of developing cartilage around the nerve will result in a nerve foramen. Figure 10 is a drawing of the scapula of a sheep embryo 5 inches in length, and is introduced here, as is also figure 11, to show how essentially similar these scapulae of other Ungulates are to that of the pig, except in this one particular, that the scapula is never at any time perforated by spinal nerves.

V. POST-NATAL SCAPULAE

From general knowledge and considerations our first idea was that this could only be a transient phase in the embryonic development. Therefore, one of the problems set for solution was concerning the manner of the exit of these nerves from the scapula.

As already stated, the work was begun upon a 25-mm. embryo in which two nerves pass through the scapula. From this a study was made of embryos of increasing size until the full-term fetus was reached. However, there was no 'transient phase,' but, much to our surprise, a steady increase from one nerve in the smallest embryo to five in the fetus just prior to birth.

This carried the problem over from prenatal to postnatal life. For this part of the work we were fortunate enough to secure a series of pig scapulae from animals ranging in age from a few days to that of the before-mentioned over-sized hog. The exact ages of some of these scapulae are unknown, but figures 13 and 14 are from young pigs of the same litter and are about one week old. Figure 20 is from an animal ten months old, and figure 21 is that of the old boar.

This series of figures (13 to 21) show only the suprascapular region, and are taken from a series of drawings of the entire scapulae, which are being incorporated into an investigation now under way on the development of the scapula, coracoid, and sternum in the pig, the mouse, and other mammals, to which paper this present one might be said to be a foot-note.

Figures 13 to 21 show conclusively that there is no exit of nerves from the suprascapula at any time in the life history. The suprascapula of an old hog (fig. 21) shows no indication of ossification and maintains the same relative proportion to the scapula as obtains in the smallest specimens examined. The presence of these nerves is correlated directly with the existence of the cartilaginous suprascapula as a permanent structure. In forms lacking a suprascapula the spinal nerves of the scapular region run in a dorsal direction on the inner side of the scapula until they gain its upper border, then, turning laterally over the edge of the bone, they descend ventrally on its outer surface a short distance, and then ramify out into their muscular and cutaneous branches.

In the pig we could obtain this same condition by removing the suprascapula. If this were done it would be seen that the nerves are perfectly regular and normal and subscribe exactly to those described just above for animals without the suprascapula. The unusual relation here between scapula and spinal nerves is due primarily not to any shifting or abnormality in the nerves themselves, but rather to the upbuilding of a large and permanent cartilaginous suprascapula around and above them.

But little more need be said concerning figures 13 to 21. That they show considerable variation in the position and number of foramina is apparent at once. This latter point is easily cleared up by referring back to figures 2 and 3. Figure 3 was obtained by gross dissection of a pig one week old and shows the spinal nerve dividing some distance before it reaches the suprascapula. Figure 2 shows two spinal nerves passing in an undivided condition through the cartilage, but separating into two branches each immediately beyond. The number of

foramina in any specimen, then, depends upon two things: first, the number of spinal nerves involved, and, second, whether they branch before or after passing through the foramina.

Figure 15 with its five nerve exits may be interpreted as having branches of three spinal nerves in the cartilage. One of these, however, divided into three rami at a point medial to the scapula. The procartilage was then laid down around them, giving us, with the other two undivided nerves, the number five, that we would expect. This contention is also borne out by the relative sizes of the foramina; there are three small ones for the branching spinal nerve and two large ones for the undivided nerves. The number five is met with twice in the material at hand, the other being in a fetus, and is the largest number observed. From this, as the figures show, we have all numbers down to one.

VI. FOSSILS

In addition to searching for these nerves among the contemporary relatives of the pig, it occurred to me that an examination of fossil remains might prove instructive and would open up the possibility of establishing another link in the phylogenetic relationships of the mammals. To this end a careful examination was made of books containing plates of fossil mammals. These included, first of all, that monumental work of Cope on the "Fossil Mammals of the Tertiary." Also such texts as Osborn's "Age of Mammals" and others were gone through. In none of them, however, was there any indication of nerve foramina. But this is perhaps, after all, not strange, for the nerve openings, when present, are always in cartilage, and this would not likely be preserved with the bony scapula.

VII. CONCLUSIONS

The observations made in the course of this investigation seem to justify the following conclusions:

1. These nerve foramina are not present in other mammals.
2. They are always found in the domestic pig, also in all other genera of the Suidae, the peccaries alone excepted.

3. The number of nerves differs, due to variation in branching and also to the size of the embryo.

4. The dorsalis or posterior primary branches of the first four or five spinal nerves are the ones involved.

5. The nerves are normal in their course and directly comparable to the same nerves in other mammals after the removal of the suprascapula.

6. The presence of the nerves in the suprascapula is brought about by the developing procartilage, which envelops the nerves.

7. The nerves never pass out of the scapula at any time in the life history of the pig.

8. The suprascapula in the pig never ossifies, but maintains the same relative proportion to the scapula throughout life.

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PLATE 1

EXPLANATION OF FIGURES

1 Sagittal section of pig embryo. All four foramina cut through in same section.

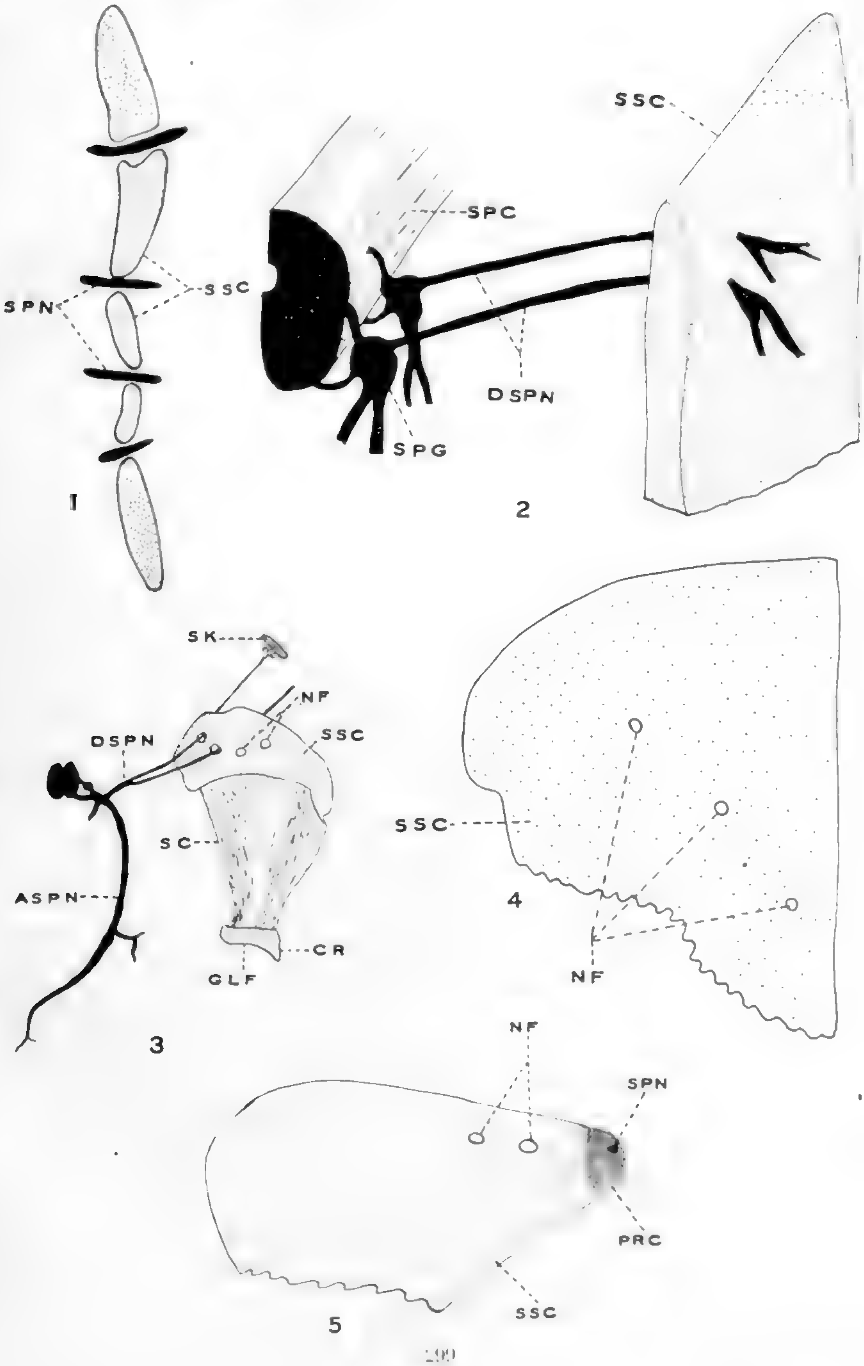
2 From a wax model of a 37-mm. pig. Only upper part of scapula is shown. These are the third and fourth spinal nerves.

3 Gross dissection of a pig one week old. This is the first spinal nerve, and its dorsalis branch could be traced distinctly from the ganglion, on through the suprascapula, and to its distribution in the skin.

4 Upper and posterior end of wax model of 47-mm. pig.

5 Suprascapular part of wax model of scapula of 27-mm. pig. Two foramina are entirely formed in the cartilage, while the procartilage is enclosing a third spinal nerve. This should be compared with figure 4, a larger embryo, where the third nerve is now surrounded by cartilage.

aspn, anterior branch spinal nerve
cr, coracoid process
dspn, dorsalis branch spinal nerve
glf, glenoid fossa
nf, nerve foramina
sc, scapula
sk, skin
spc, spinal cord
spg, spinal ganglion
spn, spinal nerve
ssc, suprascapula


SPN
SSC
1
SSC
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DSPN
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NF
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SSC
SC
ASPN
CR
GLF
3
SSC
4
NF
NF
SPN
PRC
SSC
5

PLATE 2

EXPLANATION OF FIGURES

6 Transverse section through suprascapula of 37-mm. pig, showing passage of nerve through foramen.

7 Camera drawing of transverse section of 18-mm. pig. Scapula is entirely procartilaginous, and in its growth is about to enclose a spinal nerve.

8 This is a transverse section through suprascapular region of a 35-mm. pig, showing cartilage at one end and procartilage at the other. It demonstrates very well how these nerves are surrounded by the growing cartilage.

9 Another transverse section of a 27-mm. pig to show relation of nerve and suprascapula.

10 Scapula of sheep embryo 5 inches long. Dotted line indicates what will be upper limit of bony scapula.

c, cartilage
ct, centrum
ntch, notochord
prc, procartilage
spn, spinal nerve
ssc, suprascapula

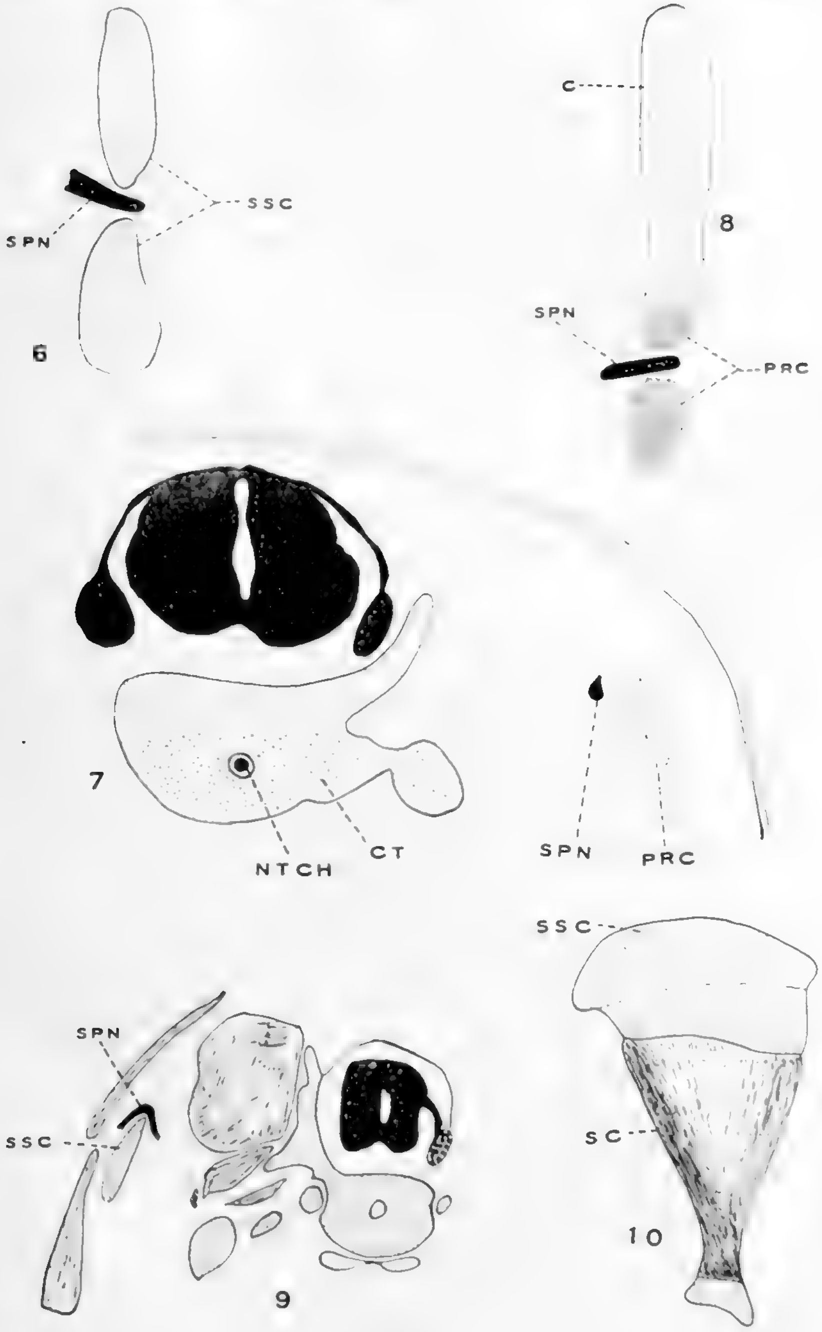
6
SPN
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PLATE 3

EXPLANATION OF FIGURES

11 Scapula of red deer showing well-developed suprascapula, but without foramina. Modified after Flower.

12 Oblique section of 27-mm. pig with foramen partly cut through.

13 Fetus approaching full term. Cartilage extends as a continuous band from suprascapula along upper margin of spine to its highest point. Three nerves pass through the cartilage in this specimen, which are undivided as in figure 2.

14 Full-term fetus showing four foramina. It is probable that these are two branches each of two spinal nerves, which divided on the medial side of the scapula. See also figure 3.

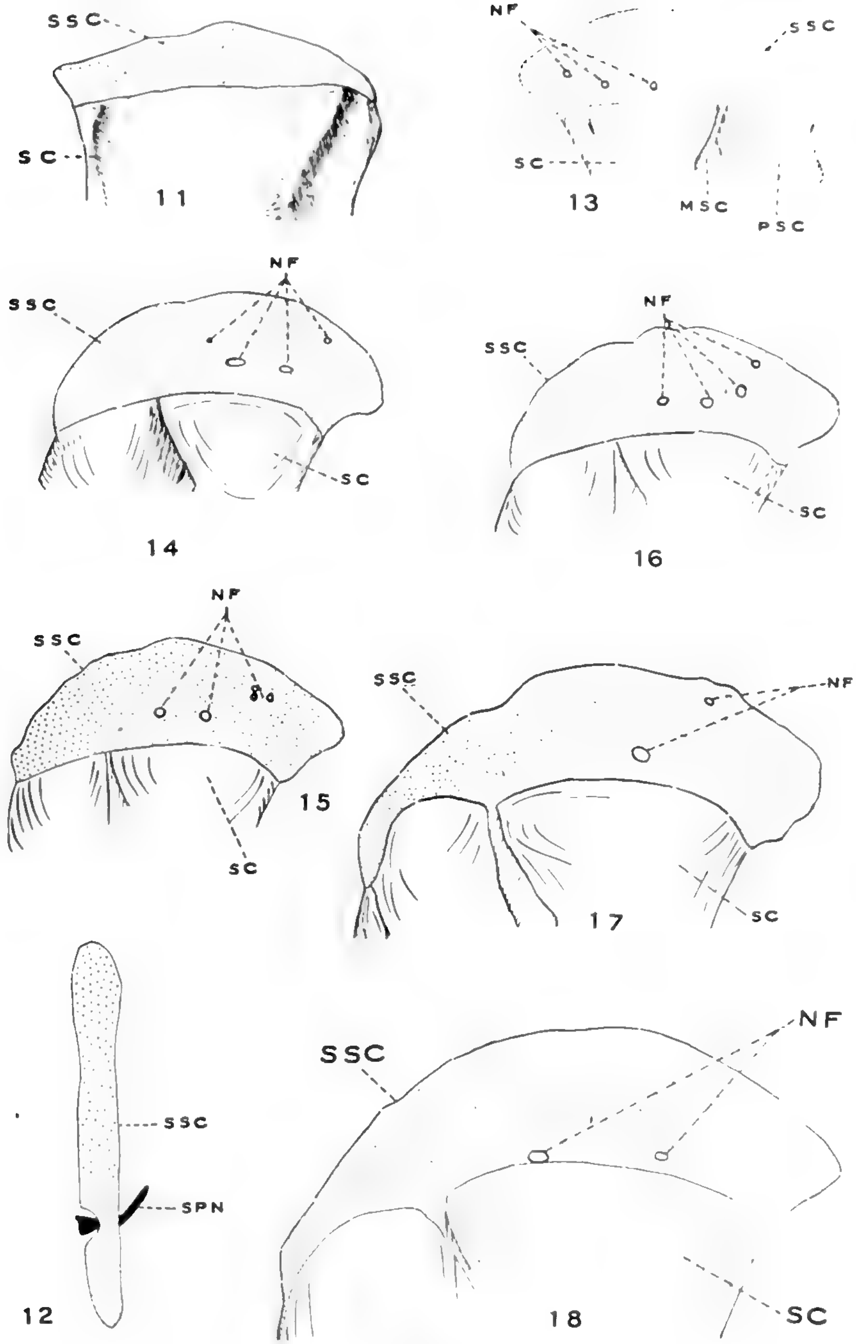
15 Pig one week old. Has five foramina, the largest number found. The three small ones in a cluster represent the rami of one spinal nerve.

16 Same age as figure 15, but is quite different as to the arrangement and size of foramina.

17 Pig several months old. This and figure 18 show the only two postnatal specimens with but two foramina.

18 Older than in figure 17. Same number of foramina, but a difference in position.

msc, mesoscapula (spine)	*sc*, scapula
nf, nerve foramina	*spn*, spinal nerve
psc, prescapula	*ssc*, suprascapula


SSC
SC
11
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15
SC
SSC
NF
SC
17
SSC
SPN
12
SSC
NF
SC
18

PLATE 4

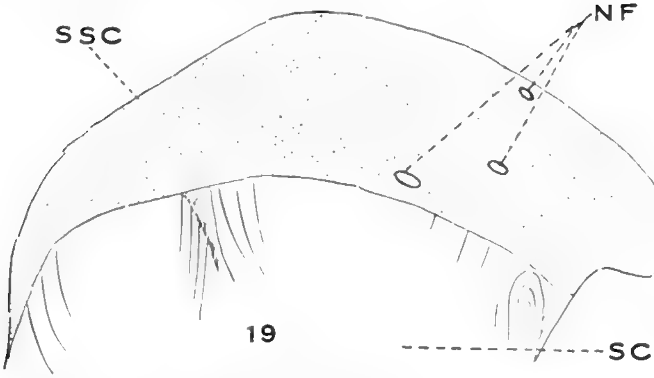
EXPLANATION OF FIGURES

19 Pig nearing young adult life. Three large foramina, indicating undivided spinal nerves.

20 Young adult pig between ten months and one year old, with three foramina.

21 This scapula is from an old boar (age unknown) which weighed 450 pounds. The suprascapula is still cartilaginous and three nerve openings are present.

nf, nerve foramina
ssc, suprascapula
sc, scapula



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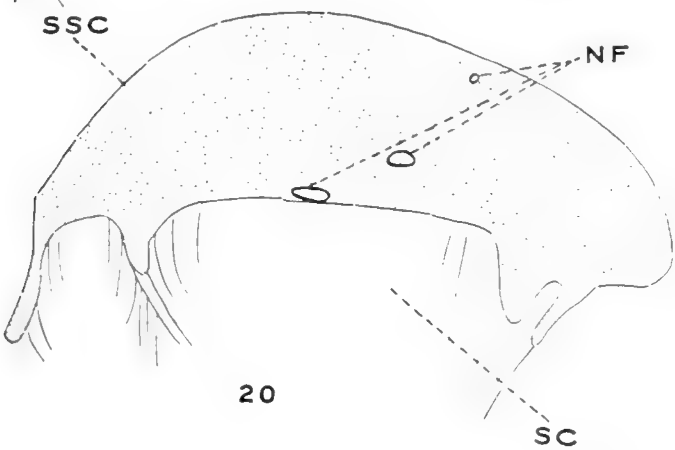

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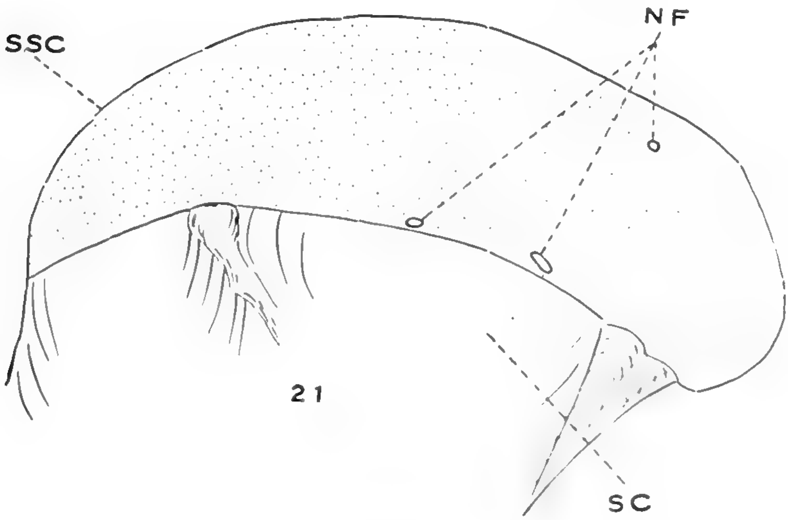

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Resumido por la autora, Florence May Alsop.

El efecto de las temperaturas anormales sobre el desarrollo del sistema nervioso del embrión de gallina.

En el presente trabajo, la autora incluye una breve discusión de los métodos empleados para producir anormalidades en los animales; también revisa algunas de las causas de las anormalidades encontradas en huevos. Las conclusiones obtenidas por el presente trabajo son las siguientes: 1) El calor excesivo y una limitada cantidad de calor producen la muerte de muchos embriones de gallina y dan lugar a varias formas de anormalidades en el sistema nervioso de otros. 2). Las temperaturas elevadas aceleran el desarrollo de los embriones, mientras que las temperaturas bajas retardan el crecimiento. 3). Las anormalidades producidas en fases tempranas del desarrollo no continuan su crecimiento en los embriones de setenta y dos horas. 4). Las temperaturas comprendidas entre los 103° y los 108°F. produjeron 90 por ciento de embriones anormales; de estas anomalias 46% aparecían en la región de la cabeza y 54% en el tubo neural. 5). En huevos incubados a temperaturas comprendidas entre los 94° y 101°F., el 67% de los embriones eran anormales; el 17% de estas anomalias aparecian en la región cerebral y el 83% en el tubo neural. 6). Los huevos incubados a la temperatura normal produjeron casi 6.5 por ciento de embriones anormales. Muchas de estas anormalidades eran distintas de las producidas por las temperaturas anormales. El trabajo contiene tres tablas, trece figuras y una bibliografia con cincuenta y tres referencias. Richard E. Scammon, author.

Translation by Dr. José F. Nonidez,
Columbia University

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, DECEMBER 23

THE EFFECT OF ABNORMAL TEMPERATURES UPON THE DEVELOPING NERVOUS SYSTEM IN THE CHICK EMBRYOS[1]

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THIRTEEN FIGURES

CONTENTS

I. INTRODUCTION

The work described in this paper was taken up as a result of finding abnormal chick embryos among the slides used in the

[1] Contribution from the Zoological Laboratory, Kansas State Agricultural College.

embryology laboratory classes.[2] Although the cause of these abnormal embryos was not known, yet they suggested the possibility of trying to produce abnormal embryos by exposing the eggs to various degrees of temperature. In the discussion which follows the writer has shown that an abnormal amount of heat may cause deformities in the nervous system of chick embryos and also that different kinds of abnormalities were produced by apparently the same condition as well as by different conditions.

II. DISCUSSION OF LITERATURE

Abnormalities in developing embryos have been produced in many ways. Some scientists have used an abnormal amount of heat, others have produced abnormalities by different chemicals, while still others have produced abnormal embryos by hybridization and centrifugal force.

A. Methods of producing abnormalities in animals

1. Abnormal heat. Among those who produced abnormalities by temperature variations is Greeley ('01), who found that by lowering the temperature of stentor certain well-defined structural changes took place which were not necessarily incidental to the permanent suspension of the vital functions of the cell.

King ('03) also has shown that excessive heat causes abnormalities in the toads' eggs and hastens development.

2. Hybridization. Loeb ('15) used low temperatures and heterogenous hybridization to produce blind fish embryos. Immediately after fertilization he put the eggs into a temperature of 0° to 2°C. and produced from 20 to 30 per cent abnormal embryos.

Newman ('17) also produced monsters through hybridization.

3. Effect of chemicals. Fere ('99) used the fumes of alcohol and ether and obtained many abnormal embryos. In an incu-

[2] I wish to express my indebtedness and appreciation to Dr. Mary T. Harman for the use of the slides collected by her and also for her criticism of this paper. I also give acknowledgement to Dr. R. K. Nabours for his interest and encouragement in this work.

bator set over the ventilator from the chemistry laboratory the eggs contained many abnormal chicks due to the fumes of chemicals.

Reese ('12) used narcotics as an agent in producing abnormalities in the development of hens' eggs.

4. Centrifugal force. Centrifugal force is another factor employed by some to produce abnormalities. Banta and Gortner ('14) produced accessory appendages in the amphibian larvae through the action of centrifugal force. Also Conklin ('11) describes abnormal results obtained by the action of centrifugal force upon the organization and development of the eggs of fresh-water pulmonates.

III. CAUSES OF ABNORMALITIES IN EGGS

Eggs that are abnormal at the time of laying probably play an important part in the development of abnormal embryos, and a certain per cent of abnormal chicks found under apparently normal external conditions are probably due to conditions through which the egg passed before it was laid. These abnormalities in the eggs may be caused by several factors, according to Parker ('06).

A. Abnormal ovary

The ovary may be abnormal or diseased. An injury may cause a breaking away of the egg from its follicle before it has ripened.

B. Abnormal oviduct

An abnormal oviduct may retain an egg until the second yolk is broken away from the follicle. In this case both yolks are sometimes encased in one shell. Schumacher ('96) explains this condition by an antiperistalsis in the oviduct. The egg retained in the oviduct has developed more than is normal at the time of laying, and the cooling of an egg at the time of the formation of the neural plates produces a large per cent of abnormalities, as will be discussed later.

C. *Abnormal ovary and oviduct*

An abnormal ovary and oviduct are sometimes present and may produce abnormal eggs.

Eyeleshymer ('07) by careful calculations has found that the temperature of the hen during the first week of incubation is from three to four degrees higher than that of the eggs under her, and if an egg is retained in an environment of 102° to 104°F. when the temperature of the eggs under a hen is found to be 98° to 100°F. it is very probable that the four degrees of extra heat will cause abnormal development. Especially was this found to be true in the first twenty-four hours of development of those eggs used in this experiment. The embryo was very susceptible to a change of a few degrees of temperature at the time when the neural tube was closing, and more so at the time when the nervous system was beginning to form.

The physiological zero, according to Lillie ('08), is that temperature below which the blastoderm undergoes no development whatever. It has been shown by Edwards ('02) that a small amount of development will take place at 21°C., and therefore he places the physiological zero between 20° and 21°C., although 28°C. is accepted by many authors as the degree of temperature below which no development takes place. Therefore, if 20°C. be accepted as the physiological zero, abnormal development or growth may be caused by variations in the time of development in the hen, or by development that may take place in warm weather after the egg is laid and before it is placed under incubation.

IV. DETERMINATION OF THE AGE OF CHICK EMBRYOS

A. *Number of somites*

The age of chick embryos is generally determined by the number of somites found in the early stages of development. This method is used by Lillie and others up to the end of the fourth or sixth day of incubation, but after this time the number of somites does not increase, and embryos are classified according to length.

B. Size of embryo

There were many difficulties involved in trying to determine the age of the embryos by the greatest cervical-caudal length. The cranial and cervical flexures appeared at different stages of development in the embryos which had been incubated for the same length of time. An egg kept in the incubator for twenty-four hours under high temperatures often developed both head flexures, while another left in the incubator forty-eight hours in temperatures below normal had in a large per cent of instances formed only the cranial flexure. In a small per cent of those eggs that had been incubated for forty-eight hours below 102°F. no flexure was formed. But in these embryos the tissue appeared to be dead, as it did not stain properly.

Abnormal flexures were present in many of the embryos. Probably the most noticeable was a bending backwards of the trunk, forming a convexity on the ventral side of the chick, which is the exact reverse of the normal development. Even the tail bud in a small number of these abnormal flexures was turned dorsally instead of ventrally.

Another condition which made it impossible to determine the age of the chicks by body length was the variation in size of the embryos which were otherwise equally developed, i.e., the number of somites was the same, the flexures were equally developed, and all had been kept in the incubator under the same degree of heat and during the same length of time. Still some were found to be 50 per cent or more larger than others. This might have been due to heredity, as it is found frequently in eggs that have been incubated in normal temperatures.

Another great hindrance to classifying the age of embryos in this experiment by either of these methods was the partial development of certain parts, for example, only one nerual plate developed in some embryos, and this development in most instances was abnormal. One side of the brain developed more than the other in a few of the embryos, while in others no brain was formed.

C. Length of time of incubation

The length of time of incubation is not generally used as a basis of classification because under the best of conditions the variations in development is sufficient to prevent close grading. But in this paper the length of time of incubation is the only method employed to classify the age of the embryos. The somite method cannot be applied because many of the chicks do not develop somites at all. This was found to be true of those eggs which were incubated at low temperatures. A few embryos showed no somites after having been in the incubator twenty-eight hours. On the contrary, a few embryos developed accessory somites. A second row was formed lateral to and alternating with those somites of each of the first two rows of somites.

These conditions all made it difficult to use any method in determining the age of the embryos produced under abnormal temperatures. Therefore, in speaking of a twenty-four- or forty-eight-hour chick in this work the writer is referring to an embryo obtained from an egg which has been in the incubator twenty-four or forty-eight hours, respectively, regardless of its degree of development.

V. METHOD OF INCUBATION

The eggs used were obtained from the Poultry Department of the Kansas State Agricultural College. Each egg was marked with the number of the hen that laid it. Also the number of her mate was recorded. These records were kept in case the history of a particular chick was needed to help solve or verify certain deformities that might appear in some of the specimens, and to determine whether or not the cause of certain types of abnormalities was in the parent stock or was caused by the method of incubation. Such data were not necessary, however, because the large per cent of abnormalities was in all probability due to the method of incubating the eggs.

Three hundred and three eggs were incubated for this experiment. This does not include those abnormal embryos collected

by Doctor Harman nor those incubated and used as a control or check upon the results obtained here.

Mr. Boyd, a student in the college, incubated one hundred and eighty-six eggs under normal conditions. He was making embryology slides for the Zoology Department, and the eggs used in his work were incubated at the same time and under the same conditions, with the exception of temperature, as those used in my work. Data were kept upon the abnormal chicks found in these eggs which were incubated at normal heat, and these results served as a control and were compared with those obtained by running the eggs at abnormal temperatures.

The eggs were all incubated in a wooden incubator, which was quite satisfactory, as the wooden frame was not susceptible, to any great extent, to a change in the temperature of the atmosphere.

The heat was furnished by a kerosene lamp. It was easily kept under control, varying not more than a degree from what was desired.

The incubators were kept in the basement of the incubating building of the poultry farm of the college. The temperature here was as cool and constant as it could be found at this time of the year, which was during the months of June and July.

The eggs were collected every hour of the day. The date and hour of laying were marked upon each shell. In no instance had the eggs been laid more than twenty-four hours before they were put into the incubator.

No eggs were incubated longer than three days, and therefore this would limit the nervous system to the early embryonic brain and neural tube. The deformities described are those found only in the brain and neural tube.

VI. METHOD OF KILLING AND FIXING

The embryos were killed and partly fixed in the egg shell. One side of the shell was cut away; the albumen was poured out and a few drops of 10 per cent nitric acid was poured upon the embryo to kill it and partially fix it. The specimen was then cut away from the blastoderm, taken out of the shell, and the

vitelline membrane and adhering yolk material were washed off with water. The embryos were now transferred to Bouin's fluid for further fixation. After one or two hours the embryos were placed in 70 per cent alcohol, which was changed several times during each of the next two or three days. Finally, when the picric acid was removed, the specimens for whole mounts were stained in alum carmine and mounted in balsam. Some of these embryos after being examined as whole mounts were dissolved off the slides, embedded in paraffin, and sectioned.

All of those embryos incubated for seventy-two hours were stained in borax carmine for sectioning. These were cleared in zylol and mounted in balsam.

VII. ABNORMALITIES PRODUCED BY HEAT

The discussion which follows takes up the different kinds of abnormalities and the per cent of abnormalities which were produced in the nervous system of chick embryos. Abnormal temperatures were the only factor taken into consideration in producing these results.

A. *The effect of low temperatures upon development*

Different variations of temperature were applied to the eggs with different results. Low temperatures were employed first. By low temperatures is meant any degree of heat between 94°F. and 102°F. (102° to 104°F. is the temperature generally considered as normal in incubating eggs.) No eggs were incubated below 94°F., because the per cent of embryos that died increased, and it did not prove satisfactory to run the incubator at a temperature below 94°F.

Edwards ('02) describes a condition observed by Warynski in which the embryo is made abnormal as a result of low temperature. He says,

> The yolk when cooled rises and presses the blastoderm against the vitelline membrane, to which it sticks. If this happens during the first two days, while the embryo is unprotected by the amnion, the pressure causes an arrest of development and consequent malforma-

tions. The exact character of these, and the region of the embryo in which they occur, cannot be predicted, since it is a matter of chance as to the part of the blastoderm which will adhere to the vitelline membrane.

The above condition was not found in any of the embryos produced in this experiment. The vitelline membrane washed off easily with no part of the blastoderm adhering to it.

The abnormality which occurred most frequently as a result of incubating eggs at low temperatures was the lack of folding in of the neural plate into the neural tube for some distance above the primitive knot. The neural folds in the anterior region generally formed, although in some cases abnormally, and these folds extended posteriorly about as far as the first somites. This condition was very noticeable in those embryos which had been incubated only twenty-four hours or less (figs. 1 and 2). Also below the region of the primitive knot the plate failed to develop for a short distance. But posterior to this place the folds, in most instances, developed normally.

At the primitive knot not only was the tube formed, but extra thickenings of the walls and extra cells, apparently of ectodermal origin, nearly filled the central canal in some embryos and closing it completely in others. In still other specimens the extra tissue filled the canal in such a way that two or three neural canals were found in one neural tube. This condition was best studied in the transverse sections of the forty-eight- to seventy-two-hour chicks (figs. 11 and 12), although it was easily traced developing in the eighteen-hour chicks. The abnormality appeared here as a mass of cells developing in the region of the primitive knot. It stained more heavily than the surrounding cells and was easily followed through the series of older embryos.

In the twenty-four-hour specimens two distinct folds were beginning to form on either side of the primitive node (fig. 1). The cells at this point were apparently able to resist the lack of heat and proceeded with development, while the cells of the neural plate for a distance anterior and posterior to this region did not multiply so rapidly, and consequently the neural folds

here were not so far developed as those in the region of the primitive node.

In the thirty-six-hour chicks the neural tube had, in most of the embryos, grown together nearly the entire length of the tube. But that part of the neural fold in which development first started stained darker than that anterior or posterior to it, showing that the tissues were thicker and that more cells had been produced in this part of the neural tube than elsewhere.

The abnormalities in the forty-eight-hour chicks did not show so distinctly as in the embryos that had been incubated a shorter length of time. Of the fifty specimens examined as whole mounts, thirty-seven appeared normal. Twelve of these seemingly normal chicks were embedded in paraffin and sectioned. The result showed eleven abnormal and one normal tube. The central canal in all eleven was either closed or partly closed with the thickening of the tube wall. The abnormality in all cases was more distinctly shown in the lumbar region than elsewhere. The canal in some specimens contained loose tissue the entire length of the tube below the hind brain.

Of the thirty embryos incubated seventy-two hours, all but one seemed normal. All of the twenty-nine sectioned transversely. One was destroyed in the process of cutting. Of the remaining twenty-eight, twenty-three had developed an abnormal neural tube, or nearly 83 per cent were abnormal. This per cent would have been larger, I think, if those embryos which had died before the seventy-two hour stage of development was reached could have been included. Nearly 23 per cent were unable to resist the low temperature or were too abnormal to live three days in a temperature which ranged from four to seven degrees below that which is found in the natural incubation of the hen.

Another abnormality produced by low temperature was the curved primitive streak (fig. 3). This was seen in the early stages of growth and developed into a tube that was abnormal in curvature (fig. 4). In the majority of these embryos the posterior third of the tube turned off to the left side instead of extending in a straight line with the anterior two thirds. This

condition was found more often in the embryo below the age of twenty-eight hours than in the older chicks; although it was found in one forty-seven-hour specimen which was no further developed than a normal twenty-four-hour chick should be (fig. 4).

A third condition which occurred in the development of embryos under low temperatures was that of one neural plate forming into a neural fold before the other began development (fig. 7). This lack of development of the neural fold appeared more often in the younger embryos than in those older. The other neural plate finally developed after a longer period of incubation, for in but a small number of forty-eight-hour chicks did one neural fold show a development in advance of the other.

B. The effect of high temperature upon development

Embyros incubated with excessive heat developed different kinds of deformities than those described above. The amount of heat used here varied between 104° to 108°F. No eggs were incubated at a higher temperature than 108°F. The excessive heat at this point caused nearly 25 per cent to die before the end of the period of incubation.

A large per cent of the abnormalities appeared in the brain region. The most conspicuous of these was a constriction of the neural tube below the optic vesicles. The extra amount of heat seemed to affect the rate of growth of some parts of the brain region more than other parts. The optic vesicles and midbrain region developed more rapidly than that part of the brain between them, hence the constriction or folding in followed (fig. 6). The neural tube posterior to the brain developed uniformly in most embryos, with the exception of a few which showed the extra development again in the lumbar region. This resembled very much the abnormality found in the same place in those embryos developed with low temperatures.

The higher temperatures had an effect upon the somites of the embryos which was the reverse of that caused by the lower degrees of heat. Nearly 4 per cent of the chicks incubated at

104° to 108°F. developed extra somites lateral to the ordinary somites (fig. 8), while in embryos produced below 101°F. the number of somites was diminished, or in some of the twenty-four-hour chicks they did not form at all. In all the embryos but one, where the eggs had been incubated longer than twenty-four hours, the somites could be distinguished. In the one exception the egg had been incubated forty-seven hours under low temperature. The neural tube was normally developed as far as could be discerned in the whole mount, but no somites could be counted.

In none of the embryos examined could the 'accessory optic vesicles' described by Locy ('97) be found. He describes them as follows:

> There exists in the brain walls of the chick and Acanthias serial differentiations of epithelium, that take the form of vesicles, closely connected with the optic vesicles, and therefore called accessory optic vesicles. These structures are very transitory—extending over a period of three hours in the chick—and they disappear before the true brain vesicles arise with which they might otherwise become confused. Their existence supports the hypothesis that the vertebrate eyes are segmental and that the ancestors of vertebrates were primitively multiple-eyed inasmuch, the optic vesicles arise before the brain vesicles, the primitive relationship of the former is not the diverticula from the latter. This condition is secondary.

The question as to what time in the early development is the embryo most susceptible to an abnormal temperature was worked upon to a certain extent, but owing to the small number of eggs used in some instances the result could not be as conclusive as it would have been if a larger number of eggs had been used.

Eggs are put into the incubator at a low temperature, and the heat was increased several degrees for different lengths of time. Others were placed in the incubator at a certain temperature and the heat was lowered several degrees. Still other eggs were incubated at a nearly constant temperature. A few of the eggs were put into the incubator at a low degree of heat, the temperature was raised and then allowed to decrease.

VIII. RESULTS

The following table shows that a variation of a few degrees of heat produced a large per cent of abnormalities as long as all variations were below normal.

Table showing effect of low temperatures upon developing chick embryos

TIME IN INCUBATOR	NUMBER OF EGGS USED	KINDS OF MOUNTS	RANGE OF TEMPERATURE	NUMBER OF NORMAL EMBRYOS	NUMBER OF ABNORMAL EMBRYOS	PER CENT OF ABNORMALITIES
72	28	Transverse	96–99–96	5	23	83
72	1	Whole	96–99–96	0	1	
47–48	50	Whole	95–101	37	13	26
48	12	Transverse section	95–101	1	11	92
27–32	42	Whole	95–101.5–97	21	21	50
26	10	Whole	98–96	0	10	100
24	25	Whole	95.5–100 102.5–99	1	24	96
24	8	Transverse section	95.5–100 102.5–99	1	7	87.5
22	6	Whole	98–96.5	3	3 slow	50
18	15	Whole	97–96.5	All slow in development		

The abnormalities produced in the twenty-four-hour chicks did not grow out to any noticeable degree in the seventy-two-hour embryos. Or, in other words, the seventy-two-hour chicks did not outgrow the abnormalities produced in them at the twenty-four-hour stage. In the above table the per cent of abnormalities is somewhat less in the seventy-two-hour chicks than in the younger embryos. But this does not take into consideration those embryos which were dead when the shell was opened. All of these that were in a condition to be examined for structure at all were abnormal. These apparently deformed conditions may have been caused by the degeneration of the cells. Yet the eggs must have been abnormal or deformed which caused the embryos to die under the lack of heat which was resisted to a certain extent by those embryos which were alive when examined.

The eggs incubated for twenty-seven to thirty-two hours are grouped together in the table. None of these embryos were sectioned, but were examined as whole mounts. The per cent

of abnormal chicks was the same as the per cent of normal chicks. But, as was shown in the case of the forty-eight-hour embryos, the per cent would no doubt prove much greater if these specimens had been sectioned.

No very satisfactory results were obtained from incubating eggs less than twenty-four hours under low temperatures. The embryos had not developed sufficiently to conclude whether they were normal or abnormal. Most of them showed a very low degree of development. A slight differentiation of cells in the primitive streak had taken place.

Table showing effect of high temperatures upon developing embryos

TIME IN INCUBATOR	NUMBER OF EGGS USED	KINDS OF MOUNTS	RANGE OF TEMPERATURE	NUMBER OF NORMAL EMBRYOS	NUMBER OF ABNORMAL EMBRYOS	PER CENT OF ABNORMALITIES
48	18	Whole	105–107	1	17	44
28	16	Whole	103–108	0	16	100
24	14	Whole	105–107.5	3	11	79-
22	11	Whole	107–107.5	2	9	82-
Total				6	52	90

Most of the embryos in this table were farther advanced in their development than an embryo ordinarily is on being in the incubator the lengths of time specified above. For example, the twenty-four-hour chicks were developed fully as much as a thirty-six hour embryo would naturally be under normal temperature (Compare figs. 5 and 7).

The result of Mr. Boyd's work showed that out of one hundred eighty-six eggs incubated fifteen were abnormal. But during his work the light in the incubator lamp accidentally burned out, producing out of the eighteen eggs being incubated four abnormal embryos. Excepting this set of eggs, there were one hundred seventy-one eggs, eleven of which were abnormal. Thus according to his work less than 6.5 per cent of the eggs produced abnormal chicks under apparently normal artificial conditions.

One interesting condition noted in the abnormal embryos which were produced under seemingly normal conditions, was that the deformities in these chicks were dissimilar to those

produced by the variations in temperature. The abnormalities found in Mr. Boyd's embryos were on the whole much like some of those noticed by Doctor Harman in the laboratory slides. A few were monsters which were different from any of those found in the laboratory or produced by artificial means.

The abnormalities produced by excessive heat were located in somewhat different parts of the embryos than those produced by a limited amount of heat. This can be shown quite clearly in the table below.

LOCATION OF ABNORMALITY	LOW TEMPERATURE	HIGH TEMPERATURE	TOTAL IN LOW TEMPERATURE	TOTAL IN HIGH TEMPERATURE	PER CENT IN LOW TEMPERATURE	PER CENT IN HIGH TEMPERATURE
Brain only	0	6	22	41	17	46
Neural tube only	83	12	105	47	83	54
Heat and tube together	22	35				

Some embryos had an abnormal brain only. In others the abnormalities were in the neural tube. While still others possessed an abnormal brain and neural tube. The formation of the neural folds into a tube that was abnormal occurred more frequently than any other condition. As shown in the table above, only six conditions of abnormality were located in the head region alone. Ninety-five embryos were abnormal in the neural tube. In fifty-seven of the embryos both the brain and the neural tube were affected.

IX. CONCLUSIONS

1. Excessive heat and a limited amount of heat produced death in many chick embryos and various forms of abnormalities in the nervous system of others.

2. Excessive temperatures hastened the development of embryos, while low temperatures retarded their rate of growth.

3. The seventy-two-hour chicks did not outgrow any of the abnormalities produced in them at an earlier stage of development.

4. Temperatures between 103° and 108°F. produced 90 per cent abnormal embryos. Of these abnormalities 46 per cent were in the head region, 54 per cent were in the neural tube.

5. In those eggs incubated at 94° to 101°F. 67 per cent were abnormal; 17 per cent of these abnormalities were in the brain region and 83 per cent were in the neural tube.

6. Incubating eggs at normal temperature produced nearly 6.5 per cent abnormal embryos. Many of these abnormalities were different from those deformities produced under abnormal temperatures.

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PLATES

All figures were drawn at table level with a no. 5 eye-piece and a 16-mm. objective, also with the aid of a camera lucida. Front lens of objective was removed for whole-mount drawings. All drawings reduced one-half.

PLATE 1

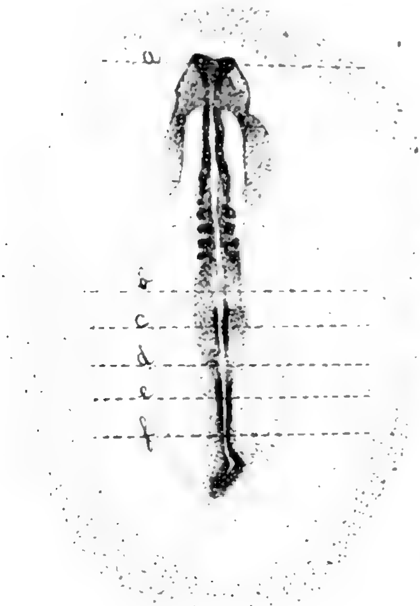
EXPLANATION OF FIGURES

1 Twenty-four-hour embryo, incubated at 95.5°F. to 98.5°F. Broken lines show plane of sections in fig. 2. *a*, through optic vesicles showing folds failing to unite; *b*, through neural plates back of fifth somite, no neural folds present; *c*, through primitive node region, neural folds formed; *d*, through neural plate posterior to primitive node region, no neural folds present; *e*, through tube forming in posterior region; *f*, through posterior region.

2 Twenty-eight-hour chick incubated at 96.5°F. to 98.5°F. Letters corresponding to those in fig. 1 indicate sections through these regions.

3 Twenty-four-hour chick with curved primitive streak at *a*. Incubated at 105°F. to 107.5°F.

4 Forty-seven-hour chick with curved neural tube at *a*. Folds failed to form at *b*. Incubated at 95°F. to 97°F.



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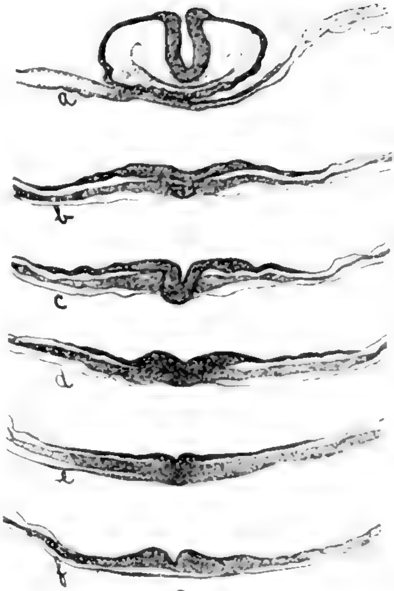

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PLATE 2

EXPLANATION OF FIGURES

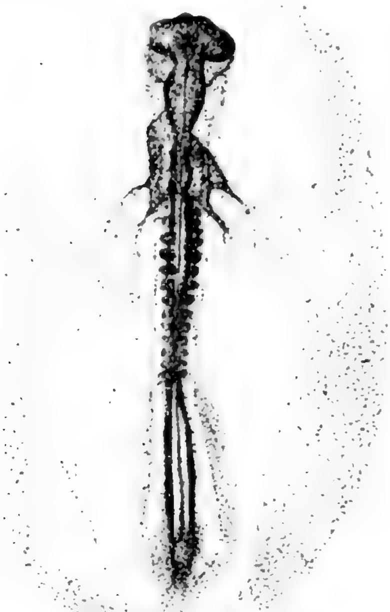
5 Twenty-four-hour chick. Incubated at 103.5°F. to 108°F. Development equal to that of normal thirty-six-hour chick.

6 Twenty-eight-hour embryo incubated with temperature at 103.5°F. at beginning of incubation, raised to 108°F. at end of fifth hour. Constriction formed below optic vesicles at *a*.

7. Twenty-four-hour chick. Incubated at 102.5°F. to 99°F. *a*, one neural fold forming in brain region; *b*, abnormal development of notochord.

8 Twenty-eight-hour embryo incubated at 103.5°F. to 108°F. *a*, accessory somites; *b*, abnormal brain; *c*, posterior limit of neural folds.

PLATE 2



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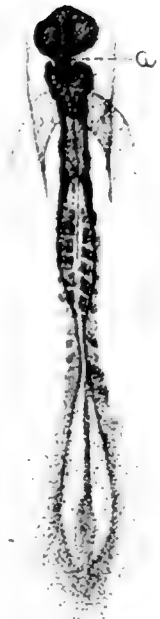

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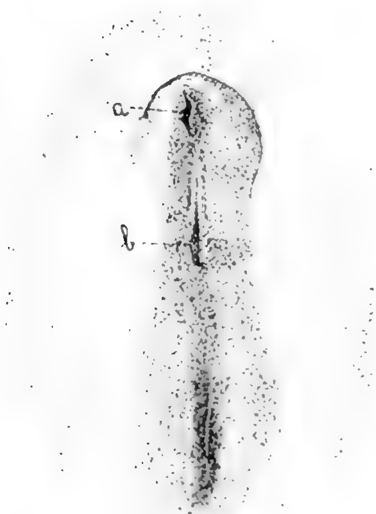

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PLATE 3

EXPLANATION OF FIGURES

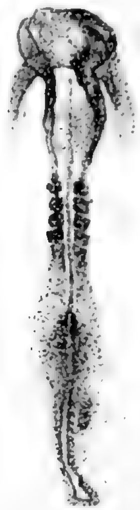
9 Twenty-nine-hour embryo incubated at 100.5°F. to 101.5°F. Neural tube in brain region not closed. No tube formed in remainder of body.

10 Twenty-two-hour embryo incubated at 107°F. to 107.5°F. Neural tube formed only in brain region.

11 Transverse section of seventy-two-hour chick through posterior part of neural tube. Incubated at 97.5°F. to 96°F. Tube showing three central canals at *a*.

12 Transverse section of same embryo. *a*, two central canals present in neural tube.

13 Forty-eight-hour chick incubated at 96.5°F. to 97°F. *a*, extra tissue in lumbar region.



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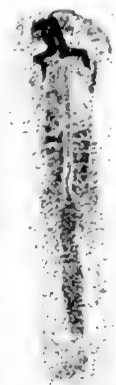

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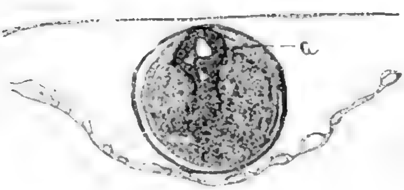

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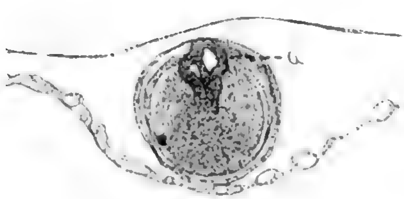

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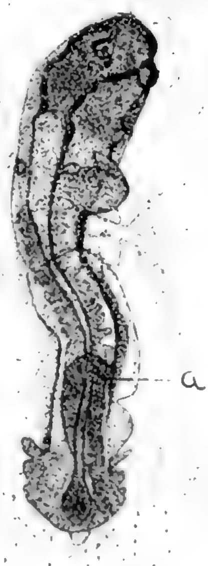

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PROCEEDINGS OF THE AMERICAN SOCIETY OF ZOOLOGISTS

SIXTEENTH ANNUAL MEETING

The American Society of Zoologists held its Sixteenth Annual Meeting jointly with Section F of the American Association for the Advancement of Science and in affiliation with the American Society of Naturalists and the Ecological Society of America, December 26, 27 and 28, 1918, in Gilman Hall, Johns Hopkins University, Baltimore, Maryland.

BUSINESS SESSION

Election of Members

At the session for transacting business, held at 5 o'clock on Friday, December 27, President George Lefevre and Vice-President L. L. Woodruff being absent, William Patten was appointed chairman for the session. The following persons, having been recommended by the Executive Committee to the Society for election to membership, were duly elected:

BAKER, ARTHUR CHALLEN, B.S.A., Ph.D. (University of Pennsylvania), Entomological Assistant, *Bureau of Entomology, Washington, D. C.*

DETWILER, SAMUEL RANDALL, Ph.B., Ph.D. (Yale), Instructor in Anatomy, Yale Medical School, *Anatomical Laboratory, Yale Medical School, New Haven, Conn.*

HUNT, HARRISON RANDALL, B.S., Ph.D. (Harvard), Assistant Professor of Zoology, West Virginia University, *Morgantown, W. Va.*

POWERS, EDWIN BOOTH, A.B., M.S. (Chicago), Ph.D. (Illinois), Assistant Professor of Biology, Colorado College, *Colorado Springs, Col.*

TALIAFERRO, WILLIAM HAY, B.S., Ph.D. (Johns Hopkins University), Second Lieutenant Medical Research Division, Chemical Warfare Service, *62 Park St., New Haven, Conn.*

ROBERTS, ELMER, B.S., Ph.D. (University of Illinois), Associate in Genetics, *University of Illinois, Urbana, Ill.*

Election of Officers

The Committee on Nominations consisting of D. H. Tennent, R. G. Harrison and M. F. Guyer, having recommended persons

for election to the various offices of the Society and no other nominations being presented, the following elections were made:

For *President*, C. M. Child, Chicago, Illinois. To serve one year.

For *Vice-President*, H. H. Wilder, Northampton, Massachusetts. To serve one year.

For *Secretary-Treasurer*, W. C. Allee, Lake Forest, Illinois. To serve three years.

For member at large of the *Executive Committee*, to serve five years, George Lefevre, Columbia, Missouri.

Report of the Secretary-Treasurer

On account of pressing duties Captain Caswell Grave, *Chemical Warfare Service*, resigned from the position of Secretary of the Society in September and W. C. Allee was elected by the Executive Committee to act in his place until the Annual Meeting. Captain Grave was able to continue to perform the duties of the Treasurer and submitted the following report:

Seven members were recommended to be dropped for non-payment of dues and two members resigned from the Society.

Financial Statement

The financial statement of the Treasurer for the year 1918 is as follows:

Receipts

Balance on hand January 1, 1918	$733.85
Back dues for the year 1916, 1 at $5.00	5.00
Back dues for the year 1916, 1 at 7.00	7.00
Back dues for the year 1917, 6 at 5.00	30.00
Back dues for the year 1917, 5 at 7.00	35.00
Back dues for the year 1917, 1 at 8.00 (foreign)	8.00
Back dues for the year 1917, 1 at 11.50	11.50
Dues for 1918, 59 at 5.00	295.00
Dues for 1918, 1 at 6.00 (Life member)	6.00
Dues for 1918, 167 at 7.00	1169.00
Dues for 1918, 1 at .50	.50
Dues for 1918, 1 at 7.97 (foreign)	7.97
Dues for 1918, 10 at 11.50	115.00
August 1, 1918, Fifth dividend, Ind'l. Sav. & L'n Co.	9.00
October 7, 1918, Interest at 4% on deposits	38.93
Total	$2471.75

Expenditures

January to December inclusive

For telegrams and telephone calls	$7.43
For express charges	27
For stationery and stamps	33.15
For typewriting	3.99
For typewriter ribbon	1.00
For printing announcements and programs	33.00
For 253 subscriptions to journals (Wistar Institute)	1570.00
For Railroad fare and Pullman, Lake Forest to Baltimore and return	69.98
For hotel expenses of Secretary in Baltimore	14.64
Total	$1733.96
Balance on hand December 28, 1918	737.79

Report of the Auditing Committee

The Auditing Committee reported as follows:

We have examined the books of the Treasurer and have found that the balance here given is correct, provided that the items in credit and debit columns have been correctly entered. Data for the verification of these items have not been presented.

(Signed) S. O. Mast,
A. Richards.

By motion the reports of the Treasurer and the Auditing Committee were accepted and the incoming officers were instructed that in the future it should be their policy to pay bills of the Society by check.

At the opening of the session Saturday afternoon, December 28, the Auditing Committee requested leave to present a new report which follows:

We have found upon reexamination of the Treasurer's books, that except for petty cash expenditures, data for the verification of all expense accounts are at hand and that the accounts are correct.

(Signed) S. O. Mast,
A. Richards.

Committee on Premedical Education

The Committee on pre-medical education appointed in 1915 and continued in 1916 was discharged without report.

Deposit of Records of the Society

On request the incoming Secretary-Treasurer was instructed to deposit the records and past correspondence of the Society in the fire proof vaults provided for that purpose by the Marine Biological Laboratory of Woods Hole, Mass.

The Secretary was instructed to extend the thanks of the Society to the officials of Johns Hopkins University and to the Local Committee for the entertainment of the Society during the meetings.

The business session adjourned at 5.30 P.M.

Session for the Presentation and Discussion of Papers

At sessions held during the afternoon of December 26 and the morning and afternoon of December 27, 18 papers were presented in full and 19 were read by title.

In the absence of the President and Vice-President, Bennett M. Allen, was made presiding officer of the Thursday afternoon session. William Patten, Vice-President of Section F presided at the morning and afternoon sessions on Friday.

The morning of December 27 was given over to a joint session with the Ecological Society of America when the following papers were presented:

W. J. Crozier, University of Illinois. Further Contribution upon the Natural History of Chromodorus Zebra: the question of adaptive coloration. A. S. Z. (Read by title.)

Edwin B. Powers, Colorado College. The Hydrogen Ion Concentration of the sea water of Puget Sound and the Reactions of the herring (Clupea Pallasii Cuvier) to Hydrogen on concentration in sea water. E. S. A.

The P. H. of Puget Sound in the vicinity of Friday Harbor varies with weather conditions, tides, depths, and locality. The

herring reacts positively to a P_H of 7.9 to 8.0. The reaction is positive to this P_H concentration both from a lower and a higher P_H.

H. H. Reed, Cornell University. The Zoological Significance of the functional fenestral plates in the ear capsule of caudate amphibia. A. S. Z. (Read by title.)

Harry C. Oberholser, National Museum. Ecological Investigations under the Federal Government (30 min.). E. S. A.

The most important ecological investigation under Federal Government auspices are carried on as a basis for other work, and are of far reaching importance. The Fish Commission studies the relation of fishes to their environment; the Forest Service that of trees; the Bureau of Plant Industry of various other plants, particularly with regard to plant diseases and plant introductions; the Bureau of Animal Industry, the life history of internal animal parasites; the Bureau of Entomology, the life history of insects in their relation to economic problems; and the Biological Survey, the relations of animals, birds, and other animals to their environment and to each other, for the determination of the life zones of distribution.

W. H. Longley, Goucher College. The Coloration and Habits of West Indian and Hawaiian reef fishes. A. S. Z.

Henry S. Pratt, Haverford College. The Distribution of the Internal Parasites of the Fish and other Aquatic Vertebrates of Oneida Lake, New York. (15 min.) E. S. A. (Read by title.)

V. E. Shelford, Illinois Natural History Survey. Suggestions as to the Climograph of deciduous forest invertebrates, as illustrated by experimental data on the codling moth. (20 min.) A. S. Z. and E. S. A. (Read by title.)

W. J. Crozier, University of Illinois. On the Nature and Source of some adaptive features in the etiology of Chiton. A. S. Z. (Read by title.)

On Saturday morning, December 28, the Society held no session but met with the American Society of Naturalists to listen to their program of papers on Evolution and Genetics.

Retiring Secretary-Treasurer

At the opening of the Afternoon Session, in addition to the report of the Auditing Committee already given, the retiring Secretary-Treasurer, Caswell Grave, was tendered the thanks of the Society for his faithful, efficient and loyal services. The motion to this effect was unanimously passed amid applause.

The Society then passed to the afternoon's program.

Conference between Government and Laboratory Zoologists.

Subject: Methods of Securing Better Coöperation between Government and Laboratory Zoologists in the Solution of Problems of General or National Importance. Professor C. E. McClung, presiding.

Paper on plans and problems of the Bureau of Entomology that can be furthered by coöperation with laboratory zoologists. Dr. L. O. Howard.

Discussion led by Professor J. G. Needham, Cornell University.

Paper from the Bureau of Fisheries. Dr. Hugh Scott.

Discussion led by Professor H. B. Ward, University of Illinois.

Paper from the Bureau of Animal Industry. Dr. B. H. Ransom.

Discussion led by Professor Herbert Osborn, Ohio State University.

Paper from the Biological Survey, Dr. E. W. Nelson.

Discussion led by Major C. A. Kofoid, Fort Sam Houston.

In the absence of Major Kofoid, Professor R. K. Nabours, Manhattan, Kans., gave a short discussion of Dr. Nelson's paper.

Dr. McClung then introduced Dr. J. C. Merriam, Vice-Chairman of the National Research Council, who outlined the tentative plans of the Council for advancing coöperative research in America and Professor McClung concluded the discussion with the explanation of the application of these plans to the problems of coöperation between Government and Laboratory Zoologists.

The proceedings of this Conference will be published in full in *Science*.

LIST OF TITLES

PARASITOLOGY

1. On the transmission of two fowl tapeworms. James E. Ackert, Kansas State Agricultural College.
2. Recent discoveries concerning the life history of *Ascaris lumbricoides*. G. H. Ransom and W. D. Foster, Bureau of Animal Industry, Washington, D. C.
3. The true homology of the cuticula and subcuticula of trematodes and cestodes. H. S. Pratt, Haverford College.

COMPARATIVE ANATOMY

4. The metamorphosis of two species of cyclops: *Cyclops signatus* (*C. Albidus Jurine*) and *Cyclops americanus* Marsh. Esther F. Byrnes.
5. The olfactory organs of Orthoptera. N. E. McIndoo, Bureau of Entomology, Washington, D. C.

GENERAL PHYSIOLOGY

6. The formation of buds 'Tethya' buds in sponges of the genus Coppatias. W. J. Crozier and Blanche B. Crozier, Bermuda Biological Station for Research.
7. On the temporal relations of asexual propagation and gametic reproduction in Coscinasterias; with a note on the function of the Madreporite. W. J. Crozier, University of Illinois, College of Medicine.
8. The olfactory sense of lepidopterous larvae. N. E. McIndoo, Bureau of Entomology, Washington, D. C.
9. Sensory reactions of *Chromodoris zebra*. W. J. Crozier, Bermuda Biological Station and L. B. Arey, Northwestern University Medical School.
10. The relative stimulating efficiency of continuous and intermittent light upon *Vanessa antiopa*. William L. Dolley, Jr., Randolph-Macon College, Ashland, Va.
11. The rates of CO_2 produced by starved and fed Paramecia and their possible relations to the rates of oxidation in the unfertilized and fertilized sea urchin egg. E. J. Lund, University of Minnesota.
12. The photoreactions of partially blinded whip-tail scorpions. Bradley M. Patten, Western Reserve University, School of Medicine.
13. Excretion crystals in ameba. A. A. Schaeffer, University of Tennessee.
14. The reactions and resistance of certain marine fishes to H. ions. C. E Shelford, University of Illinois.
15. A simple method for measuring the CO_2 produced by protozoa and other small organisms." E. J. Lund, University of Minnesota.
16. The effect of KCN on the rate of oxygen consumption of Planaria. George Delwin Allen, University of Minnesota (introduced by E. J. Lund).
17. The influence of temperature and concentrations on toxicity of salts to fish. Edwin B. Powers, Colorado College (Introduced by V. E. Shelford).

ECOLOGY

18. Further contributions upon the natural history of *Chromodoris zebra;* the question of adaptive coloration. W. J. Crozier, University of Illinois, College of Medicine.
19. The zoological significance of the functional fenestral plate in the ear capsule of caudate amphibia. H. D. Reed, Cornell University.
20. The coloration and habits of West Indian and Hawaiian reef fishes. W. H. Longley, Goucher College.
21. Suggestions as to the climograph of deciduous forest invertebrates as illustrated by experimental data on the codling moth. V. E. Shelford, Illinois Natural History Survey.
22. On the nature and source of some adaptive features in the ethology of Chiton. W. J. Crozier, University of Illinois, College of Medicine.

EMBRYOLOGY

23. The anlage of endoderm and mesoderm in the opossum. Carl Hartman, University of Texas.
24. The oestrous cycle in rats. J. A. Long, University of California.
25. Results of extirpation of both thyroid and pituitary glands in tadpoles of Bufo and Rana (5 minutes), Bennett M. Allen, University of Kansas.
26. Miscellaneous notes regarding experimental studies upon the endocrine glands of Rana and Bufo (10 minutes), Bennet M. Allen, University of Kansas.
27. Effect of the extirpation of the thyroid gland upon the pituitary gland in Bufo. Mary Elizabeth Larson, University of Kansas (introduced by Bennet M. Allen).

EVOLUTION AND GENETICS

28. The solitary and aggregated generations in Salpidae. Maynard M. Metcalf, Orchard Laboratory.
29. Correlation of fertility and fecundity in an inbred stock. Roscoe R. Hyde, Indiana State Normal School and Johns Hopkins University.
30. The extent of the occurrence of sex intergrades in Cladocera. Arthur M. Banta, Station for Experimental Evolution.
31. Nuclear reorganization and its relation to conjugation and inheritance in Arcella vulgaris. H. M. MacCurdy, Alma College.
32. Several ways in which Gynandromorphs in insects may arise. T. H. Morgan, Columbia University.
33. Duplication. C. B. Bridges, Columbia University. (Introduced by T. H. Morgan.)

EXHIBITS

1. Demonstration of sex intergrades in Cladocera. A. M. Banta, Station for Experimental Evolution.

2. Models showing typical stages in the development of the human embryo. Department of Embryology, Carnegie Institution of Washington.

ABSTRACTS

1. *On the transmission of two fowl tapeworms.* James E. Ackert, Kansas State Agricultural College.

In studying the life cycles of fowl cestodes the writer recently demonstrated experimentally that the house fly, *Musca domestica* L., may transmit to chickens a tapeworm which appears to be *Davainea tetragona* (Molin 1858) Blanchard 1891. Chicks hatched in an incubator were reared in a screened experimental feeding house. The cement floor and walls (eighteen inches high) exclude all worm-like animals and the vestibule facilitates in eliminating any winged forms.

The feed of the chicks is carefully inspected and free from animal tissues except occasional feedings of fresh beef. Under such conditions experimental and control chicks have been kept continuously for more than four years. Frequent intestinal examinations of control chicks during this period have never yielded a single parasitic worm.

The flies were trapped at local poultry yards in which spring chickens were found by examination to be infested with tapeworms. The live flies were immersed in tap water and permitted to dry, after which the house flies, *M. domestica*, were sorted out, one by one, and given in small numbers to young chicks. Occasional movements of the flies indicated that any larval tapeworms in them probably were unaffected by the immersion. Between September 23, and October 19, 1918, several thousand *M. domestica* were given to seventeen young chicks reared in the experimental feeding house. Four of these chicks have been examined to date. The results from chicks 232 and 243 examined November 1, 1918, were negative, no parasites having been found, but the intestine of Chick 235 (November 4, 1918) contained ten mature tapeworms which have the characteristics of *Davainea tetragona*. Two tapeworms, obviously of this same species, were removed on November 4, 1918, from the intestine of Chick 250.

In a similar experiment (1917) the fowl cestode, *Davainea cesticillus* (Molin), was transmitted to chickens. An account of the experiment, including a description of the tapeworms transmitted, is being published in the current number of the Journal of Parasitology.

2. *Recent discoveries concerning the life history of Ascaris lumbricoides.* B. H. Ransom and W. D. Foster, Bureau of Animal Industry, Washington, D. C.

Major Stewart (I. M. S.) has lately recorded the results of investigations on *Ascaris lumbricoides* which necessitate revision of former conceptions of its life history. The present writers have repeated and supplemented Stewart's experiments, confirming his main results but reaching different conclusions.

Ascaris eggs after incubation when swallowed hatch in the intestine. The larvae within a short time after hatching can be found in the liver and portal vein. Reaching the lungs in the circulation, they undergo considerable development within a few days. Via trachea and esophagus they reach the intestine and develop slowly to maturity if the animal infested is a suitable host. Otherwise (rat, mouse, guinea-pig, rabbit) they soon pass out in the feces without further development, and quickly die. Stewart's view that rats and mice act as intermediate hosts of the *Ascaris* of man and pig is untenable. Partial development of these parasites in these animals is an expression of incomplete adaptation to strange hosts, and not a phase of the normal life cycle.

Ascaris and related forms may bear a causal relation to lung troubles of obscure origin in children, pigs, and other young animals. The larvae can cause fatal pneumonia in pigs.

In lambs and young goats the pig *Ascaris* can develop much further than in rats, mice, etc., and may reach a stage approaching maturity. Very young pigs appear more susceptible to infection than older pigs. *Ascaris* eggs injected subcutaneously will hatch, the larvae migrating to the lungs and developing thereafter as in infection per os.

3. The true homology of the cuticula and subcuticula of trematodes and cestodes. H. S. Pratt.

Most textbooks of zoology teach that the cuticula of trematodes and cestodes is homologous to that of arthropods and annelids and is secreted by the subcuticula which is thus homologous to the hypodermis. I presented facts in Volume 43 of the American Naturalist which tend to show that no such homologies exist, but that both cuticula and subcuticula belong genetically to the parenchyma and are consequently mesenchymatous and not extodermal structures. I now present an additional proof of this position. A cystocercous cercaria, *C. fusca*, recently studied, possesses prominent wart-like protuberances on its tail. These lie outside of the layers of longitudinal and circular muscle fibers and subcuticular cells, and are composed exclusively of the characteristic tissue forming the parenchyma of the worm bounded on the outer surface by the general cuticula. The subcuticular cells thus do not enter the protuberances and cannot secrete their cuticula, which is as in all trematodes and cestodes, simply the peripheral portion of the parenchyma.

4. The metamorphosis of two species of cyclops: C. signatus, (C. albidus Jurine) and C. americanus Marsh. Esther F. Byrnes.

There are nine stages in the metamorphosis of both species. The typical nauplius with three pairs of appendages molts. The second stage has a fourth appendage. *The second molt* produce a third nauplius with the fifth and sixth appendages indicated. *The third molt* produces a typical cyclops with six antennal segments. The mouthparts are present as in the adult. Rami of the swimming feet are unsegmented. The *fourth molt:* There are seven antennal segments.

Rami of the first and second feet are two-jointed. The third foot is unsegmented and the fourth indicated. *The fifth molt:* There are nine antennal segments. Rami of first, second and third feet are two-jointed; The fourth foot is unsegmented and the fifth foot is present. *The sixth molt:* There are ten antennal segments. All the feet are two-jointed. The fifth foot is fully developed. *The seventh molt:* There are eleven antennal segments. All rami are three-jointed as in adult. Abdomen immature. *The eighth molt* produces an adult with seventeen antennal segments.

C. americanus shows a marked irregularity in the jointing and armature of the feet in stages *6* and 7. This irregularly suggests an explanation of the wide variation found in the armature of the adults of the viridis type. C. signatus shows no corresponding variations.

Elongation of parts is by intercalation of segments.

During the progressive development, adult characteristics that appear early in the metamorposis undergo no modification during later stages.

Duration of metamorphosis varies from several to 10 weeks.

5. The olfactory organs of Orthoptera. N. E. McIndoo, Bureau of Entomology, Washington, D. C.

This investigation is a continuation of my study on the morphology of the olfactory pores of insects. Both sexes of twenty-one species, belonging to twenty genera and representing the six families, have been examined. The immature stages of Blatella and Melanoplus have also been studied. Olfactory pores were always present on the legs and antennar; usually on the wings (if present), abdominal segments, cerci, head and all the mouthparts; and sometimes on the ovipositor. Relative to the antennae, olfactory pores are present on only the first and second segments; this is the first time that I have seen these organs on the antennae of adult insects, except a few at the bases of the antennae of the honeybee and of a certain weevil; nevertheless, they are common to the antennae of all larvae yet examined. They are more widely distributed in Orthoptera than in any other order yet studied; the number of them on the wings is comparatively few, while the mouthparts are abundantly supplied with them; the number on the antennae varies considerably, although fifty is a common number for an antenna.

In distribution and external structure, these olfactory pores resemble the lyriform organs of spiders more than do the same organs in any other order yet examined. They are generally oblong, sometimes almost slit-shaped, but the eye-shaped type is the most common. Some of the pore borders are radially striated; this is the first time that I have observed this type of border on adult insects.

6\. *The formation of buds ('Tethya'-buds) in sponges of the genus Coppatias.* W. J. CROZIER and BLANCHE B. CROZIER. Bermuda Biological Station for Research.

A sponge which we shall describe under the name Coppatias millbrooki, sp. nov., has been found to produce buds very closely simulating the well-known Donatia-buds ('Tethya'-buds). Bud-reproduction has not hitherto been discovered in this genus, which is toxonomically akin to Donatia; in fact, although 4 marine sponge 'genera' have been reported to form buds, of several types, the characteristic buds of Donatia have occupied a relatively unique position.

Hence it is curious that C. millbrooki inhabits Mangrove creeks harboring likewise 3 well-differentiated types of Donatia, all reproducing in the way usual for this genus, namely by means of buds. Each of these budding sponges, which occur in great profusion, has a more or less definite propagative season. The one species of Donatia found at Bermuda but not occurring in the mangrove creeks—D. lyncurium, which lives under stones on exposed shores, has never been found to produce buds in this region, although this habit is exhibited by it in other regions (e.g., in the Mediterranean).

7\. *On the temporal relations of asexual propagation and gametic reproduction, in Coscinasterias; with a note on the function of the madrepore.* W. J. CROZIER, University of Illinois, College of Medicine.

Asexual propagation of Coscinasterias tenuspina by spontaneous division of the body into two parts, commonly comprising 3 and 4 rays respectively, is at a minimum during the several months preceding, and during the actual period of, gametic reproduction (Jan.–Feb., at Bermuda); and at a maximum during the summer season midway between the sexual periods. The two methods of multiplication practiced by this species are therefore supplementary in temporal incidence as well as in kind.

The formation of new rays at a division-surface is frequently accompanied by the appearance of new madrepores. The number of madrepores is positively correlated with the total number of rays, in such a way as to suggest some kind of functional significance attaching to this relation. It is also suggested that a deficiency in this relation might be implicated in determining the onset of self-division.

8\. *The olfactory sense of Lepidopterous larvae.* N. E. McINDOO, Bureau of Entomology, Washington, D. C.

Since all larvae are more or less selective in regard to their food, it has been assumed that they can smell, although, so far as known to the writer, no experiments have been performed to prove this statement, and no one has discovered olfactory organs in them, except the ones called olfactory pores described by the writer in the June number of the Journal of Morphology.

The following larvae were used in the experiments: Tent caterpillars, fall webworms, tussock-moth larvae, armyworms and larvae of

Papilio polyxenes. The following sources of odors employed and the average reaction time of the above larvae to them are: Oil of peppermint, 14.6 seconds; oil of thyme, 9.5 seconds; oil of wintergreen, 17.6 seconds; leaves of pennyroyal, 20.1 seconds; leaves of spearmint, 22.9 seconds; wild cherry leaves, 42.5 seconds; fresh grass, 19.1 seconds; honey, 51.1 seconds; protruded glands of above Papilio larvae, 22.8 seconds; and as a control—a clean and empty vial, 60 seconds (totally negative).

In making a comparative study of the disposition of the olfactory pores, thirty species, belonging to twenty-eight genera and representing twenty families, were used. Olfactory pores were invariably found on the epicranium, front, antennae, all the mouthparts, trochanters, tibiae; usually on the tarsi; and sometimes on the first thoracic segment, last abdominal segment and on the last prolegs. The total number of pores varies from fifty-seven to eighty-four with sixty-nine as an average. In structure they are similar to those in most adult insects.

9. *Sensory reactions of Chromodoris zebra.* W. J. Crozier, Bermuda Biological Station and L. B. Arey, Northwestern University Medical School.

Differentiated receptive mechanisms mediating reactions to tactile, chemical, and shading stimulation, to the constant intensity of light, and perhaps to heat, induce local responses through the agency of peripheral, nonsynaptic, nerve nets, which in the gill plumes and perhaps in other parts exhibit decided polarization. Reactions of parts distant from the site of local activation involve central, ganglionic, synaptic transmission.

The nudibranch is, probably through the eyes, positively phototropic. The branchial collar is also sensitive to light, which causes the gill plumes to be extended. The gill plumes react, variably, to shading. Sexually mature individuals are negatively geotropic. A temperature of 31–32°C. induces negative reactions. The 'rhinophores' are directive organs for negative rheotropism in strong currents. Vibrations transmitted through the water are not responded to. Chemotropic responses are important for conjugation. Locomotion is mainly muscular, and is accomplished by the lateral margins of the foot which sucks locally. The positive stereotropism of the anterior end of the foot is responsible for righting.

10. *The relative stimulating efficiency of continuous and intermittent light upon Vanessa antiopa.* William L. Dolley, Jr., Randolph-Macon College, Ashland, Va.

Further investigation of the reactions of Vanessa antiopa in intermittent light shows that at certain flash-frequencies the stimulating effect of intermittent light is greater than that of continuous light of equal illumination; at other flash-frequencies it is less than that of continuous light; and at still others it is equal to that of continuous light. The intermittent light used was of an illumination of 3.5 m.c. and was

produced by a rotating sectored disk with one-fourth removed. At a flash-frequency of 20 per second 7 out of 10 insects reacted more strongly to intermittent light than to continuous light of equal illumination. At flash-frequencies of 2 and 5 per second 100 and 80 per cent respectively of the insects tested reacted less strongly to intermittent light than to continuous light. At flash-frequencies of 10, 30, 40, 50, 60, and 100 per second most of the insects tested reacted equally strongly to intermittent and to continuous light. These experiments consequently show that the stimulating efficiency of intermittent light depends upon the flash-frequency and that it may be greater, equal to, or less than that of continuous light.

11. *The rates of CO_2 production by starved and fed Paramecia and their possible relation to the rates of oxidation in the unfertilized and fertilized sea urchin egg.* E. J. Lund, University of Minnesota.

Feeding a starving Paramecium with yeast or yolk of hen's egg increases the rate of CO_2 production by the cell from two to three times, thus confirming previous published results on oxygen consumption. This acceleration of the oxidations occurs in the *absence of cell division.* The process of cell division, as such, in all probability is not associated with any marked change in the rate of oxidations. These results are so closely parallel to the conditions obtaining in unfertilized and fertilized sea urchin eggs that it becomes highly probable that the acceleration of the oxidations subsequent to fertilization of the echinoderm egg is due to the fact that the yolk of the egg becomes available for assimilation by the living protoplasm of the egg during the act of fertilization, and in this way results in increase of speed of oxidation similar to that in a fed paramecium.

12. *The photoreactions of partially blinded whip-tail scorpions.* Bradley M. Patten, Western Reserve University, School of Medicine.

Reaction measurements previously made on normal whip-tail scorpions (Patten, 1917) were used as a basis of comparison for measurements made on partially blinded animals subjected to the same conditions of illumination. The change from the normal reaction induced by the covering of a photoreceptor was taken as an index of the effectiveness of the photoreceptor prevented from functioning.

Each of the photoreceptors (median eyes, lateral eyes, and cutaneous sensitive areas) was eliminated unilaterally, and bilaterally; singly, and in combinations with other receptors.

All animals in which the receptive mechanism was left in a functionally asymmetrical condition exhibited, when subjected to bilaterally balanced illumination, deflections toward the side which had been made less sensitive. The amplitudes of the deflections were proportional to the degree of unbalance which had been produced in the photoreceptive mechanism.

Animals in which the receptive mechanism was left in a symmetrical condition showed an undisturbed balance of reaction when subjected

to the action of equal opposed lights. Under lateral or anterior illumination, amplitude of the deflection was reduced in proportion to the extent of the interference with the receptive mechanism.

By comparing the changes from the normal reaction induced by elimination of the various photoreceptors, their relative effectiveness can be approxmated as:

median eyes: lateral eyes: cutaneous sensitive areas: 1: 1.6: 2.2.

13. Excretion crystals in Ameba. A. A. SCHAEFFER, University of Tennessee.

Nearly all species of ameba contain visible crystals in the endoplasm. In most species, if not in all, the crystals are surrounded by a vacuole; they do not lie in contact with the protoplasm. The crystals are nearly always optically active as indicated by the polariscope, though sometimes they are not. The shape and size of crystals in amebas are of the first importance in species determination. The composition of the crystals still remains uncertain. They are probably an excretory product of some sort. They are not excreted to the outside and they do not seem to be dissolved once they are formed. After the crystals are once formed they seem to remain for a long time within the ameba. Very rapidly dividing *Amoeba proteus* have very few crystals while those dividing slowly have many crystals. Those that do not divide for six to ten days become stuffed with crystals. Several individuals of *Amoeba discoides*, a species closely related to *proteus*, that did not divide for thirty days became so loaded up with crystals that they were quite opaque and locomotion was accomplished only with difficulty. Crystals may thus have two possible fates: they may be dissolved again—for which there is no evidence, or once formed they remain so long as the ameba lives.

14. The reactions and resistance of certain marine fishes to H ions. V. E. SHELFORD, University of Illinois.

The more sensitive marine fishes react to differences between a pH of 8.1 and 8.2 produced by the addition of a very small quantity of H_2SO_4 to sea water, at one end of a gradient tank. This takes place in a manner which indicates an ability to distinguish differences in pH of 0.025. Some species select the higher H ion concentration; others select the lower, according to their habitat relations. As the H ion concentration is increased above the optimum the fishes become less able to distinguish differences; the reaction to a difference such as that between pH 7.0 and 7.2 is less definite than that indicated above. The Pacific herring reacts negatively to 0.8 part per million of sulfurous acid (H_2SO_3) in a manner which indicates an ability to distinguish 0.6 part per million. In this case the difference in H ion concentration is very slight, probably too slight to be distinguished. Reactions to other chemicals indicate that small amounts of other ions may predominate over small H ion concentration. A light increase in H ions above neutrality (pH 6.85) is fatal to herring. Other fishes are more resistant; the flat fishes are remarkably so.

15. *A simple method for measuring the CO_2 produced by protozoa and other small organisms.* E. J. LUND, University of Minnesota.

The apparatus consists of a wide mouthed glass stoppered bottle of about 100 cc. capacity, from the stopper of which is suspended a small flat stender dish containing the organisms. On the bottom of the bottle is placed a known quantity of weak $Ba(OH)_2$ for absorbing the CO_2 from various sources in the bottle. With properly arranged controls the CO_2 produced by the organisms may be determined.

Tests in which were used known quantities of Na_2CO_3 showed that with careful manipulation one is able to determine to within about 5 per cent error, a quantity of CO_2 equal to that set free from one milligram of Na_2CO_3 by an acid. Many duplicate determinations may be made during the same period of time, and temperature accurately controlled by immersion of the bottles in a constant temperature bath.

16. *The effect of KCN on the rate of oxygen consumption of Planaria.* GEORGE DELWIN ALLEN, University of Minnesota. (Introduced by E. J. Lund.)

The oxygen consumption of Planaria was measured by the Winkler method, and it was found to be reduced by 0.0002 molecular KCN to less than 30 per cent of the normal. The amount of the reduction varied with the concentration of the cyanide. Weaker concentrations, however, caused proportionately greater reduction than stronger concentrations.

The rate of oxygen consumption in a cyanide solution was practically the same during periods of different length from 2 to 36 hours.

The action of the cyanide was reversible in that worms recovered their normal rate of oxygen consumption rapidly and completely after removal from the cyanide solutions.

Worms in a solution of 0.000076 molecular KCN absorbed only half as much oxygen per gram body weight as worms that were made inactive by cutting off the heads.

Cyanides reduce the rate of oxidations in Planaria, therefore, by 50 per cent or more, independently of their action on muscular and ciliary movement.

17. *The influence of temperature and concentration on the toxicity of salts to fish.* EDWIN B. POWERS, Colorado College. (Introduced by V. E. Shelford.)

The effect of temperature on the toxicities of the chlorides of lithium and ammonium does not follow van't Hoff's rule in its entirety. The relative toxicities of lithium chloride at different temperatures to the goldfish follow very closely the square root of relative standard metabolism of the goldfish as given by Krogh. A close approximation of the relative deleterious effect of obnoxious substances to fish can be determined by comparing the constants of the equations of the theoretical velocity of fatality curves of the fish when killed in these substances. The resistance of the fish to these substances can be determined by the

same method since the resistance of the fish is the reciprocal of the deleterious effect of the substance on the fish. The relative toxicities of NaCl, $MgCl_2$, $CaCl_2$, and $BaCl_2$ to the blunt-nosed minnow (*Pimephales notatus* Raf.) are not the same as the relative conductance of these salts.

18. Further contributions upon the natural history of Chromodoris zebra: the question of adaptive coloration. W. J. Crozier, University of Illinois, College of Medicine.

This species is available for observation, and in great numbers, during most of the year. Size and character of pigmentation vary considerably. Several significant features of the color-variation have been measured. At certain periods as many as half the individuals collected have been found to exhibit extensive injuries, probably inflicted by fishes; these injuries are rarely fatal. Less serious are the smaller injuries encountered upon the mantle-margin. The gills, also, which vary in coloration, are frequently found to have been bitten. The origin of such injuries through the bites of fishes has been watched.

These facts have made possible the quasi-quantitative study of a situation unique in its importance for the theory of animal coloration, surpassing in critical value that known in any other species where of the coloration has been attentively examined. It is found, for example, that although the brilliant "yellow" element in the pigmentation of C. zebra contains from 15-65 per cent orange with 25-35 per cent yellow, in different specimens, the incidence of the several types of injury is in no way correlated with the intensity, or the manner of distribution, of the yellow pigment. Similar quantitative comparisons of the frequencies of injury for each of the several kinds of gill-coloration, and of mantle-margin pigmentation, lead to results of a character agreeing with this conclusion.

In the light of other phases of this investigation, it must therefore be considered that C. zebra is neither invisible, nor for any reason inaccessible, to animals which might inflict damage upon it. The chief value of the facts recorded lies in their being independent of any human notions relative to the concealing or revealing function of the coloration of C. zebra. It is demonstrated that although variations appropriate for the natural elimination 'less adapted' types of pigmentation is present, and although a conceivably 'selective' agent, the biting of fishes, is known to be at work, no one of the modes of coloration is in fact more immune than another.

The conclusion from this study is in agreement with my other researches on this topic: the coloration of C. zebra has no 'warning' significance, neither is it 'concealing;' it is not homochromic upon natural backgrounds; the animals themselves provide evidence, independent of the investigator's ideas, which shows that an efficient repugnatoral mechanism is possessed by this nudibranch, but that its coloration may not legitimately be regarded as adaptive either in its origin or in its present significance.

19. The zoological significance of the functional fenestral plate in the ear capsule of caudate amphibia. H. D. Reed, Cornell University.

The manner in which elements combine to form the definitive fenestral plate in the Tailed Amphibia suggests a division of this order into two legions each with its own particular morphologic type of fenestral structure. One legion includes the Ambystomidae, Cryptobranchidae, Salamandra, Sirenidae, Triton and Diemictylus. The other includes the Necturidae, Amphiumidae, Typhlomolgidae, Plethodontidae, and Desmognathidae.

The perfected apparatus could have been useful only in a terrestrial environment. This indicates that all living forms have passed through a pronounced terrestrial period and those which are now aquatic are secondarily so. It is interpreted that others are gradually changing to an aquatic abode possessing already a long larval period while others still have never experienced a regressive radiation and exhibit in structure and the suppression of the larval stage a more perfect harmony with terrestrial existence.

20. The coloration and habits of West Indian and Hawaiian reef fishes. W. H. Longley, Goucher College.

The coloration of fishes and the habitual relation they sustain to their environment are correlated upon the same terms in the West Indian region and in Hawaii. Their fixed colors, with the exception of red, repeat the dominant color notes in their surroundings, and their transient color phases are demonstrably induced by the nature of the places into which they move. There is evidence too that patterns, no less than colors, are displayed according to system. When, for example, a fish may appear in either a cross-banded, a longitudinally striped, or a self-colored phase, there is marked tendency for the first-mentioned to appear when the individual is at rest, and one of the others when it moves, or is about to move.

In displaying their colors and patterns as indicated scores of species of fishes conform, it seems safe to say, to a natural system of camouflage, whose principles are capable of experimental demonstration, for the simple reason that the creatures possess the ability to respond visibly to the tests to which they may be subjected. Appreciation and formulation of these principles would place naval camouflage, for example, upon a scientific rather than an empirical basis.

The new observation that some fishes change their coloration as they rise vertically, and leave the bottom and its influence, supplements the knowledge that they commonly change their appearance as they move horizontally from surroundings of one sort to those of another. Differences in position of comparatively few inches may be followed habitually in some species by definite changes in coloration. It is not improbable then that colors or patterns appearing in some species during the breeding season alone do not differ in function from those displayed at other times by the same species under different conditions. By as much as this is true, "nuptial coloration" is an index of changed location for a

period, and except that it may be evoked by internal changes dependent upon the sexual cycle may bear no more intimate connection with the process of reproduction.

Pictures will be shown illustrating some characteristic differences in habit on the part of fishes, the extent and character of their color changes in nature, and the limited possibility of securing pictures at present showing their surroundings in their natural colors.

21. *Suggestions as to the climograph of deciduous forest invertebrates as illustrated by experimental data on the codling moth.* V. E. SHELFORD, Illinois Natural History Survey.

The climograph (a graphic expression of the relation of an animal to temperature and humidity or evaporation in combination) of animals belonging to different climates may be expected to show characteristic differences. The results on the codling moth show a wider range of humidities which give successful emergence of pupae, at lower than at higher temperatures. Temperatures above 89.5° F. retard development; temperatures below 58° F. give proportionately more rapid development than temperatures above 58° F. The shortest pupal life for any constant temperature is usually at the lowest evaporation or the highest humidity, and the time at the higher evaporations and lower humidities is usually less than the maximum. Air movement may modify the length of the pupal stage 20 per cent. In general the climograph is oblique in the same manner as that for man as shown by Taylor.

22. *On the nature and source of some adaptive features in the ethology of Chiton.* W. J. CROZIER, University of Illinois, College of Medicine.

A discussion of some progressive modifications in the habits of Chiton tuberculatus as its age advances, involving: advantageous adjustments in the matter of food supply and the operations of feeding; certain features of coloration and appearance; a probably significant degree of assortive fecundation with respect to size (age); and the mechanism whereby the fertilization of the eggs of older females is insured. With particular reference to the way in which these aspects of the life of chiton are interconnected, and their dependence upon the heliotropism of these animals as influenced directly by environmental disturbances.

23. *The anlage of entoderm and of mesoderm in the opossum.* CARL HARTMAN, The University of Texas.

In blastocysts of about 60 cells (about 24 hours) certain cells in the formative half of the egg grow in size, round up and migrate into the cavity of the vesicle. These are the entoderm mother cells and form the anlage of the entoderm. They multiply and flatten out against the inner surface of the embryonic ectoderm.

When the vesicle has attained a diameter of 1.5–1.8 mm., the mesoderm begins to proliferate (beginning of the sixth day of gestation, days before parturition!) The axis of the embryonic area is indicated before the appearance of the mesoderm by the thinning of an eccen-

trically or posteriorly situated patch of ectoderm (seen as a light field by transmitted light). The first mesodermal cells can be recognized with certainty in the opossum egg because the embryonic area in this form consists at this stage of a single layer of cells. The first mesodermal cells migrate down out of the ectoderm in a roundish group in the mid-sagittal plane of the embryonic area behind the light field just mentioned. The group soon elongates and the anlage of the primitive streak is indicated. Their origin is strikingly similar to that of the entoderm: both germ layers arise by migration of cells from the undifferentiated superficial layer. The preparations show that the entoderm makes no contribution to either the primitive streak or the head process.

The paper is illustrated by a series of photographs of the eggs in the living state, of surface views and of sections.

24. *The oestrous cycle in rats.* J. A. Long, University of California.

The length of the cycle averages very nearly five days. It is marked by changes in the vaginal and uterine mucosae and by the liberation of eggs from mature follicles. In the vagina at the end of the dioestrum the mucosa thickens greatly as the result of mitoses (stage 0); the outer cells become stripped off exposing a cornified layer which is dry and lusterless (stage 1); the cornified cells become loosened to form a slight amount of cheesy substance (stage 2); stage 3 is marked by the advent of leucocytes, the disappearance of the cornified cells and the desquamation of the deeper non-cornified cells which together with the leucocytes characterize the dioestrum. The musoca is now moist and glistening.

The uterus, besides exhibiting changes in its mucosa, during stage 0 and the first part of stage 1 becomes greatly distended by the secretion of clear fluid (in which sperm become very active) which diminishes toward the end of stage 1. Copulation takes place during stage 1. Ovulation occurs at the end of stage 2 or at the beginning of stage 3. The uterine mucosa is regenerated at least in part by mitosis of its cells.

Suckling may delay the second ovulation following parturition about 40 days. The first ovulation follows the opening of the vagina by about a day or two. During the first few weeks following puberty the cycle is longer, 9 to 17 days.

The cycles following infertile copulations are usually 10 to 19 days long. Stimulation of the cervix of the uterus by merely inserting a glass rod during stage 1 prolongs the next cycle to 11 to 19 days! It is suggested that the vaginal plug acts in this mechanical way.

25. *Results of extirpation of both thyroid and pituitary glands in tadpoles of bufo and rana.* (5 minutes.) Bennett M. Allen, University of Kansas.

Tadpoles from which the first beginning of both thyroid and pituitary glands had been extirpated, developed in precisely the same manner as do those from which the pituitary glands alone have been re-

moved. Eight are still living eight months after removal of these glands. They show the same color changes observed in tadpoles from which the pituitary gland has alone been removed. The development of the hind limbs takes place at the same rate and to the same degree as in tadpoles from which either the thyroid or pituitary gland alone has been removed. The germ glands develop in proportion to the size of the body.

26. Miscellaneous notes regarding experimental studies upon the endocerine glands of rana and bufo. (10 minutes.) BENNET M. ALLEN, University of Kansas.

1. Numerous tadpoles upon which removal of the thyroid gland had been attempted, metamorphosed tardily and at an abnormally great size. In these, more or less imperfect thyroid glands were found. One giant thyroidless Rana pipiens tadpole transformed, one year after operation, into an unusually large frog (31.1 mm. body length) 27.9 per cent longer than the average length (24.3 mm.) of ten newly metamorphosed controls.

2. Two Bufo larvae and one Rana, all operated for removal of the hypophysis, showing the characteristic light color produced by successful removal, transformed at a body length well below normal. Each contained an imperfect hypophysis but fairly well developed thyroid gland.

3. Pituitaryless Rana tadpoles were placed in solutions of Parke Davis' Pituitrin 0 mixed with water in the proportion of 1 to 200, 1 to 1000, 1 to 2000, and 1 to 4000. In spite of this, the tadpoles showed their characteristic color change at the usual time interval after operation.

4. The writer, in collaboration with Miss Mary Larson, fed the anterior lobes of the pituitary glands of cattle to thyroidless tadpoles. The experiment was begun June 22nd and is still being carried on. These tadpoles show no greater tendency to metamorphosis or size increase than do other thyroidless tadpoles.

5. The parathyroid glands of thyroidless Bufo tadpoles were measured and found to be much larger than the parathyroids of normal controls, both of corresponding stages and of newly metamorphosed toads. This is still markedly true when allowance is made for differences in body size.

27. Effect of the extirpation of the thyroid gland upon the pituitary gland in bufo. MARY ELIZABETH LARSON, University of Kansas. (Introduced by Bennet M. Allen).

James B. Rogers '17 arrived at the conclusion that the pituitary gland continues to develop when the thyroid gland is extirpated and the anterior lobe reaches a larger size actually and relatively than in normal specimens. It was felt desirable to test this conclusion in a different amphibian type and also to make a study of the pars intermedia and the histology of the gland.

Last spring over five hundred thyroid glands were removed. One hundred and eighty brains of the thyroidless and control specimens were dissected out and measured.

The specimens were paired according to body length. They varied from five to twenty-nine millimeters. Not only was it found that the anterior lobe increased in size in the thyroidless specimens but that the pars intermedia as well grew larger in comparison with their respective controls. The five millimeter tadpoles already showed the effects of the removal of the thyroid gland. Increase in the size of the gland increased with the growth in body length. The graph will show this fact. In the thyroidless Bufo which measured from five to about twelve millimeters the pars intermedia arched around the anterior lobe while in the control the pars intermedia tended to lie in a straight line. This fact was not nearly so evident in older thyroidless specimens.

The larger thyroidless specimens were paired with still larger controls. The following average measurements for ten pairs of the larger specimens will show distinctly the increase in size of the different lobes:

	CONTROL	THYROIDLESS
	mm.	*mm.*
Average total length	22.5	42.4
Average body length	22.5	18.1
Average fore leg length	13.0	
Average hind leg length	27.7	4.5
Average vertical diameter of anterior lobe	0.3714	0.6822
Average horizontal diameter of anterior lobe	0.4001	0.6640
Average length of pars intermedia	0.7458	1.0234
Average width of the right part of the pars intermedia	0.1361	0.3124
Average width of the left part of the pars intermedia	0.1386	0.2837

An actual diminution takes place in the size of the pituitary gland at the time of metamorphosis.

There is a distinct difference in the histology of the normal and thyroidless pituitary glands. The nuclei of the thyroidless pituitary are somewhat larger than the nuclei of the control pituitary and in almost every case the nuclei of the control are angular and wedge shaped while in the thyroidless they are almost spherical. The control gland presents a very compact appearance while the thyroidless one is quite loose in texture.

Further study will be made upon the histology of the gland.

28. *The solitary and the aggregated generations in salpidae.* MAYNARD M. METCALF, Orchard Laboratory.

The comparative study of all species of *Salpidae* shows a gradual modification in the evolution of the members of the family from the Cyclosalpas to the true Salpas *(sensu strictu)* along one line, and along another line from the Cyclosalpas to the *Oligomyaria.* The modification is evidenced very clearly in the condition of the muscles, of the gut, and of the nervous system, especially the eyes.

Arranging the several species in order according to the degree of divergence, in these several regards, from the Cyclosalpas and comparing with one another the solitary and aggregated forms of each species, one sees that, in the evolution, the aggregated generation is the first to respond to the modifying influences, whatever they may be, and the solitary generation is more conservative. In most species the solitary form shows more archaic character, the aggregated form a more divergent structure. However, in the most highly evolved species, namely, the higher *Oligomyaria*, even the solitary forms have reached a condition very divergent from that of the Cyclosalpas. The aggregated form leads in the evolution, but the solitary form, at the end of the series of species, becomes almost equally modified.

Is not this wholly natural? The Salpa life cycle may be expressed as

. Egg x sperm
Embryo
Solitary forms
Stolon with buds
Aggregated form with eggs and sperm

The aggregated zooid is therefore the latest form in the ontogeny and might naturally be expected to be less conservative than the solitary form which is an earlier stage in the ontogeny. This is but another instance of a very familiar general principle.

29. Correlation of fertility and fecundity in an inbred stock. Roscoe R. Hyde, Indiana State Normal School, Johns Hopkins University.

Over 95 per cent of the eggs isolated from a mating of the wild Drosophila ampelophila gave rise to mature flies. On inbreeding the fertility rapidly declined. The fecundity of the female was not affected in this way. The correlation between the number of eggs which a female lays and the percentage which give rise to mature flies is very low. This would seem to indicate that the sterility as it affects the female bears no causal relation to reduced fertility.

30. The extent of the occurrence of sex intergrades in Cladocera. Arthur M. Banta, Station for Experimental Evolution.

Sex intergrade strains of Simocephalus vetulus have been reared in the Laboratory for three years (65 generations). These all came from the offspring of a single individual. Notwithstanding careful microscopic examination of thousands of individuals of all the laboratory strains (15) of this species, particularly during the last 20 months, no other sex intergrades have been found either in the strain which produced them originally or in any of the other strains of Simonephalus.

About 20 months ago sex intergrades were found in one of the strains of Daphnia longispina and from these we have propagated sex intergrade strains for some 36 generations. During the next few months sex intergrades were found (sparingly and only after the microscopic examination of thousands of individuals) in all except one of the six strains of

this species under cultivation. Sex intergrade strains derived from three distinct strains of this species are being propagated. Two or three sex intergrades were also seen in a strain of this species in 1915 but no young were secured from them.

Long and continued search of great numbers of individuals of 18 strains of Daphnia pulex, 7 strains of Simocephalus serrulatus, and of 11 strains of three species of Moina has not revealed a single sex intergrade individual. Hence in these species as well as in Simocephalus vetulus the occurrence of sex intergrades is apparently a rare phenomenon. Sex intergrades are relatively rare in Daphnia longispina as well, although laborious search has revealed them, mostly a single individual to a strain, in five of six strains. Once established, however, intergrade strains continue indefinitely the production of sex intergrades.

In the literature there is, presumably, only a single mention of the finding of sex intergrades (R. de La Vaulx). In view of the large number of workers with Cladocera and the extensive experimental work on this material the fact that there has been apparently only a single occurrence of sex intergrades in other laboratories speaks further for the restricted occurrence of these interesting sex forms.

31. Nuclear reorganization and its relation to conjugation and inheritance in Arcella vulgaris. H. M. MacCardy, Alma College.

The data from pedigreed cultures of Arcella vulgaris maintained from Sept., 1917, to Aug., 1918, have given the following conclusions:

1. A given individual produces a limited number of daughter cells The number varies from none to twenty-seven (the highest found).

2. These daughter cells and in turn their offspring behave in a similar way with the exceptions indicated.

3. After a period of fairly regular successive vegetative divisions, a period of 'depression' occurs. Some of the features marking this period are: reduced activity (feeding, locomotion, division), 'Punctate' shells, 'empty' shells, increased mortality. These are incidental, not essential.

4. Individuals passing successfully through this period may give rise to a new line unlike that from which it came—a marked change in size, for example. This is a 'mutation.' On the other hand, the new may be like the old line. A new period of vegetative divisions sets in and continues until another period of depression is reached.

5. While some members of a line are 'depressed,' others conjugate.

6. In pedigreed cultures of exconjugants the two members of the pair tend to produce the same numbers of daughter cells. This is in agreement with the fission rate of exconjugants in Paramoecia (Jennings).

7. In lines derived from exconjugants, after a period of vegetative divisions, individuals pass again into another period of depression, when the changes noted above and (or) conjugation may be repeated.

8. Preparations of cells made during 'depression,' and of conjugating cells show remarkably similar conditions of both chromidial net

and nuclei. Old nuclei are broken up and new nuclei are formed. This is the period of Nuclear Reorganization. This may occur within a single individual or through conjugation of two individuals. (In both permanent and temporary mounts.)

9. The inheritance of size shows changes at these periods in individual lines.

10. The following modifying factors should be mentioned: Cultural conditions influence the procedure—unfavorable conditions appear to hasten 'depression' and very favorable conditions, to delay it. The different nuclei do not always divide at the same time or pass through similar stages together. There is also evidence to show that the essential change may occur with no great break in the usual course of events, and the new arise almost or quite imperceptibly.

(The complete results are being prepared for the printers.)

32. *Several ways in which gynandromorphism in insects may arise.* T. H. Morgan, Columbia University.

Gynandromorphs have appeared in Drosophila 3 times in 16,637 flies; 32 times in 42,409; 2 times in 4,979 and 3 in 24,000; thus in the ratio of 1 to 2200. There is evidence that nearly all of them start as females; 19 were more female than male; 14 were half male half female; and 6 were more male than female. Practically all the cases found are demonstrably due to elimination of one sex-chromosome soon after fertilization. A few call for other chromosomal relations. Rarely one may even have begun as a male, but nearly all cases supposed at first to belong to this category have proved to be due to mutation in the sex-chromosome. All cases of hybrid Gynandromorphs found in bees can also be explained by the theory of chromosomal elimination. A few cases in Drosophila seem to be explicable only on the assumption ofa bi-nucleated egg, and this explanation is the only one found so far that will give a consistent explanation of Toyama's two Gynandromorphs in the silkworm moth. Bi-nucleated eggs have been described by Doncaster in other moths.

33. *Duplication.* C. B. Bridges, Columbia University.

In Drosophila melanogaster several cases of abnormal inheritance are accounted for by the assumption that in each case a piece of chromosome has been taken from its normal position and joined to another chromosome.

In the first of these cases a section of the X-chromosome, including the loci for vermilion and sable, became detached from its normal location in the middle of the X-chromosome and became joined on to the 'zero' end (spindle fiber) of its mate. For certain loci this latter chromosome carries two sets of genes—those present in the normal location and also the duplicating set. If a male carries the recessive genes for vermilion and for sable in the normal loci and the wild-type allelomorphs in the duplicating loci, he is wild-type in appearance precisely as though he were an XX female heterozygous for vermilion

and sable. A female having one such chromosome and a normal chromosome carrying the vermilion and sable genes is triploid for these loci. It has thus been proved that true recessive genes may dominate one dominant. A female tetraploid for these loci can be made, and by this means it was shown that two recessives are recessive to two dominants. Criss-cross inheritance of the Abraxas type can be initiated in Drosophila by crossing one of the above wild-type females to a vermilion sable mate, for the daughters are vermilion sable and the sons wild-type.

In another case of duplication the duplication piece contains only the locus for sable as far as known. In both of these cases the duplicating piece is joined on at the zero end (spindle-fiber), and experiments can be made in which the linkage of vermilion and sable will indicate a locus at zero instead of at 33 and 43, respectively.

A third case is the transposition of a piece of the second chromosome to the middle (spindle fiber) of the third chromosome. The genes of this duplication piece show linkage to both the second and the third chromosome at the same time. In this third case both the duplicating fragments attached to the III chromosome and the II chromosome that suffered deficiency are on hand. Any gamete that receives this deficient II chromosome dies unless at the same time it receives the third chromosome carrying the missing piece.

The most significant bearing of these cases is upon the idea of evolution of chromosome groups.

Resumido por el autor, Joseph M. Thuringer.

Anatomía de un cerdo dicéfalo (Monosomus diprosopus).

La anomalía que se describe en el presente trabajo se pretó en un cerdo a término que pesó 575 gramos. El cuerpo y cuello aparecen normales: pruebas de una fusión del esqueleto comienzan a aparecer en la séptima vértebra cervical, haciéndose más distintas hacia la cabeza. Esta presenta una cara dercha, otra izquierda y una cara compuesta con dos ojos alocados en una órbita común. Los dos hocicos estan bien separados, los orificios nasales y las bocas se abren en una naso- y oro-farínge comunes. Una lengua rudimentaria situada en el techo de la farínge oblitera toda la cavidad de esta. Las lenguas derecha e izquierda, las mandibulas y las glándulas salivares de las cara compuesta están también fusionadas. Una arteria carótida azygos, derivada de las arteria carótida común izquierda, susministra sangre a la cara compuesta. Existen dos ejes cerebrales casi normales que están unidos entre si en la médula oblonga. Una duplicación bilateral completa existe en los nervios craneales, desde el primero al octavo par. El aparato auditivo de la cara compuesta presenta un caso único de fusión.

Translation by Dr. José F. Nonidez
Columbia University

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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THE ANATOMY OF A DICEPHALIC PIG, MONOSOMUS DIPROSOPUS

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TEN FIGURES

Of the dicephalic monsters on record, the majority are either human or of the calf. Detailed descriptions which would be of much value in classification or for statistical purposes on the subject of teratology are rather few in number.

Since the publication in The Anatomical Record of The Anatomy of a Double Pig, by Eben Carey, and The Anatomy of a Two Headed Lamb, by Albert M. Reese, I came in possession of a pig belonging to the dicephalic autosites, subdivision *monosomus diprosopus.*

A diener in the laboratory of the school of medicine of the University of Alabama, who was instructed to empty the contents of a museum jar, which was in an advanced state of decomposition, made the find and brought the specimen to the writer. It presented a number of features which were deemed worthy of recording.

TOPOGRAPHICAL ANATOMY

The monster was a pig foetus (Fig. 1) apparently full term, covered with black and yellow hair. Its length 24.5 cm. and weighing 575 grams.

The body and neck appeared perfectly normal, both ventrally and dorsally, there being no duplications or deviations in proportions between the right and left halves.

The frontal aspect of face (fig. 2) showed two snouts which, excepting the mandibles, were nearly normal and symmetrical in appearance, exhibiting the usual number of teeth found at birth.

The mandibles, however, diverged to such a degree that proper closure of the mouths was impossible (fig. 3).

The two faces were symmetrical and fused in such a manner that in the 'middle'[1] (the region between the two heads), the angles of the mouths met. The two 'middle' eyes of the compound face were connected through the ocular conjunctiva; the right eye of the left face, as well as the left eye of the right face, occupying a common orbit, with one fused inferior and two obliquely placed superior palpebrae.

Between the lower eyelid of the two 'middle' eyes and the 'middle' angles of the mouths there was a small orifice concealed in a whorl of hair, which I took to be the everted united ducts of the 'middle' parotid glands, but which subsequently turned out to be the fused external auditory canals. A bristle introduced into this orifice is shown in figure 2, *duct*.

In the occipital aspect, the line of fusion terminated posteriorly in an elevation circular at the base and about 4 mm. high. This was on a line with the two fully developed right and left lateral ears and represented the 'middle' rudimentary ears. Caudad to this point the transverse diameter of the occipital region and the neck diminished very rapidly to normal.

INTERNAL ANATOMY

Much difficulty was encountered in the removal of the skin, as the macerated underlying structures showed great tendency to break off with it.

The thyreoid and thymus glands were normal, the former well meriting its name.

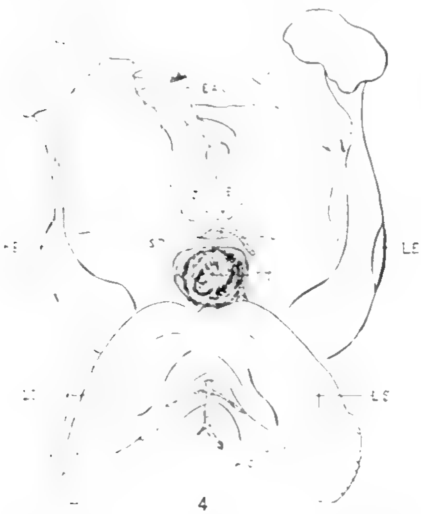
The compound face presented a large fused and horseshoe-shaped parotid gland, with each of the two vertical or free portions lying in a fossa formed by the 'middle' surfaces of the mandibles and a bony plate projecting from the junction of their angles. Independent ducts led from the free limbs of the gland to the right and left mouths.

[1] 'Middle,' hereafter used for sake of brevity in speaking of structures in the region of the fusion of the two heads.

Fig. 1. Right lateral view of entire specimen.
Fig. 2. Ventral aspect of face.
Fig. 3. Submaxillary region. *S.G.*, fused submaxillary gland; *G.H.*, genio-hyoid muscles.

The fused external auditory canals continued slightly to the left under cover of a curved bony plate to disappear beneath the fused zygomatic arch (fig. 4).

The 'middle' submaxillary glands, also fused, presented an irregular, somewhat twisted mass, the greater portion of it lying under cover of the body of the parotid gland and separated from the latter by the myelohyoid muscle.



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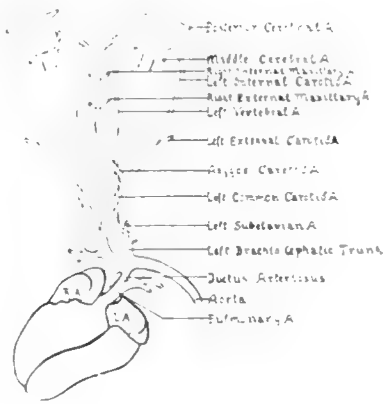

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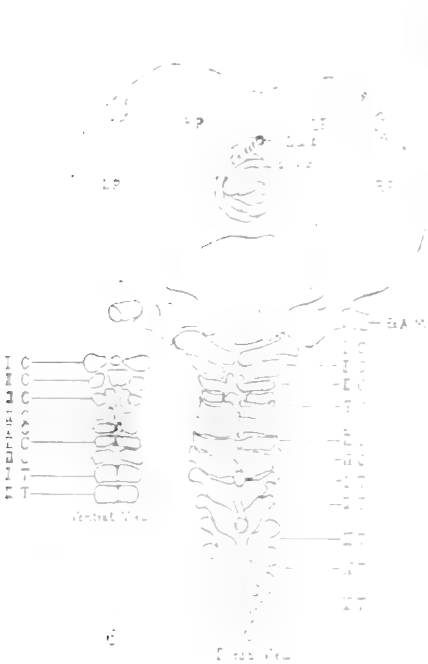
Fig. 4 Oral cavity. *E.A.C.*, external auditory canal; *P.*, plate of bone surrounding it; *J.*, proximal portion of compound joint; *S.P.*, soft palate stretched from palatine process of right to that of left side; *A.C.*, azygos carotid artery, severed; *R.E.*, *L.E.*, right and left orbits; *R.T.*, rudimentary tongue; *L.D.* and *L.S.*, right and left tongues.

Fig. 5 Schematic arrangement of circulation.

The suprahyoid group of muscles was arranged in a peculiar way. The 'middle' digastric and stylohyoid muscles were wanting. Of those attached to the 'middle' portions of the mandibles the following was noted: the right myelohyoid extended from the myelohyoid line of the right mandible toward the median line of the body and was fused with that of the left. The fibers of all four geniohyoids, excepting the most lateral ones, either united or decussated in the median line and were inserted subsequently into the bifid body of the hyoid bone.

In removing the skin covering the rudimentary ears a small duct appeared directly behind the line of fusion of the 'middle' parietal bones (fig. 6).

Oral and pharyngeal cavities (fig. 4). The alveolar processes of the 'middle' superior maxillae in their posterior one-half turned gradually toward the median line of each oral vault



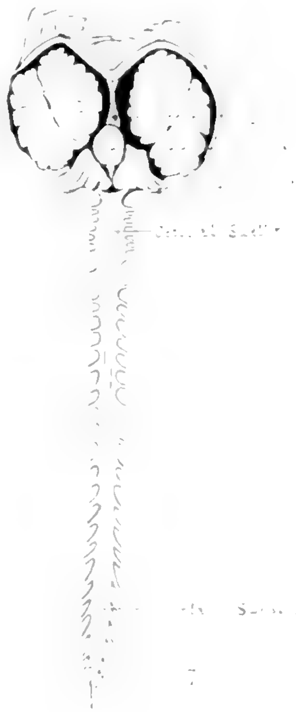


Fig. 6 Bones of head and neck. *R.P.', L.P.'*, right and left 'middle' parietal bones; *R.P.*, *L.P.*, right and left lateral parietals; *Ex.A.M.*, external auditory meatus; *L.F.*, left frontal bone.

Fig. 7 Dorsal view of cerebrospinal axis.

producing a corresponding constriction of the 'middle' posterior nares.

The posterior nares opened into a large common nasopharynx. A globular mass covered with closely set fungiform papillae and attached to the roof of the pharynx was found to obstruct practically the entire cavity of the naso- and oropharynx.

making respiration impossible (RT, fig. 4). This structure proved to be a rudimentary tongue.

The two tongues were fused at their base, their lowest point of junction, corresponding to the true median line of the body, was the last visible trace of fusion in the respiratory and digestive systems. The single epiglottis was connected to the raphe of the compound tongue in the identical manner as found when only one tongue is present, namely, by a single glosso-epiglottic fold presenting on either side a glosso-epiglottic fossa.

CIRCULATORY SYSTEM

The heart presented nothing unusual (Fig. 5). The intra-ventricular septum was complete. The two atria communicated through the foramen ovale. The ductus arteriosus was also patent.

A right and left brachiocephalic trunk was given off from the arch of the aorta. A single stem originating from the right brachiocephalic trunk gave rise to the two common carotid arteries. The other branches from this source were the right subclavian and the right vertebral arteries and two minor branches. The left brachiocephalic trunk had but three branches, the left subclavian and left vertebral arteries and a small superior cervical vessel.

The left common carotid artery gave rise to a large azygos branch opposite the cricoid cartilage. This vessel (Azygos Carotid, fig. 5) supplied the 'middle' region of the two faces, taking the place of both internal and external carotids in this region. The right and left lingual and maxillary arteries of the middle region were all derived from this single vessel.

In the 'middle' temporal fossa the azygos carotid divided into two branches, which disappeared through an opening corresponding to the foramen lacerum, to take part in the formation of the two circles of Willis, as the internal carotids of the right and left sides of the middle region.

There was no visible anastomosis between the vertebral arteries, each one passing below its respective pons as the

basilar artery to divide at a higher level into the posterior cerebrals (Fig. 5).

Only one large vein was present in the middle region which drained the submaxillary portion of this region and emptied into the left external jugular vein just above a level corresponding to the origin of the azygos carotid artery. There were but the two usual external jugular veins.

SKELETON

Caudad to the first dorsal vertebra the entire skeleton was normal. Even the cervical vertebrae on superficial examination in the wet specimen presented no great variation from the normal excepting the increased transverse diameter as they approached the skull. Figure six represents their appearance in the dried specimen after the cartilaginous portions had shrunk away from the osseous. The duplication of parts, to be seen in the figure, is no doubt due to the fusion of right and left integers.

The frontal, superior maxillary, temporal processes of the zygomatic, temporal, parietal, great wings of sphenoid, occipital bones and mandibles of the right and left heads were fused in the middle region (figs. 3, 4, and 7). Of these, the temporal bones were modified to a greater degree than any of the others and deserve special mention. No trace was found of anything simulating the mastoid portions of the two 'middle' temporals. The articular portions, from which normally extend the zygomatic processes of the temporal bones, were united and formed a crescentic articular groove (J, fig. 4). The zygomatic processes, and perhaps a small part of the articular plates as well, formed a curved plate which covered the fused external auditory canals (P, fig. 4 and Bony Plate, figs. 9 and 10). This plate formed a compound joint by articulating proximally with the articular groove just mentioned and distally with the fused condyloid processes of the 'middle' rami of the mandibles. The development of the petrous portions was restricted to the development of the semicircular canals and the cochleae of either side. These were united and arranged in relation to the middle and external ears as indicated in figures 9 and 10.

The cranial vaults were incomplete along the line of union. The right and left dura forming the only partition between the two cerebral hemispheres in the temporo-occipital region.

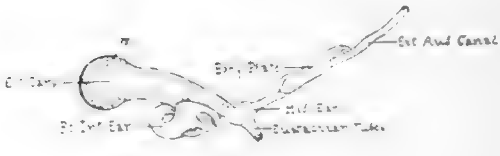


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Fig. 8. Ventral view of cerebrospinal axis showing fusion.
Fig. 9. Right side of fused auditory apparatus.
Fig. 10. Left side of fused auditory apparatus.

CENTRAL NERVOUS SYSTEM

There were two separate cerebri, two cerebelli and two fused medullae oblongatae. The right and left lateral cerebral hemispheres and the corresponding lateral halves of the midbrains, pontes, and the fused medullae oblongatae were normal in size.

An abnormally large lateral ventricle was found in the right lateral cerebral hemisphere. The right and left lateral cerebellar hemispheres, also the median cerebellar lobes (vermes), were normal.

The right and left 'middle' cerebral hemisphere diminished somewhat in size in the occipital region. From this point caudad a gradual diminution of the 'middle' halves of the mid-brains, pontes, and medullae oblongatae was observed. The 'middle' cerebellar hemispheres were entirely absent. Fusion of the two cerebrospinal axes was effected in the lower part of the fourth ventricle and was limited to the medulla oblongata.

Complete bilateral duplication of the cranial nerves, from the first to the eighth pair existed. The 'middle' glossopharyngeal nerves united to form a single trunk which terminated in and around the rudimentary tongue. Caudal this point no duplicate cranial nerves were found.

AUDITORY APPARATUS

Most of the uncertainty about the genetic significance which the various parts of the fused auditory apparatus presented cleared up upon their removal and dissection. Figure 9 is a sketch of the right side of the intact structures. Figure 10 shows the left side of same with the united external ears incised and everted. The ossicles formed a conglomerate bony mass in the centrally located dilated portion corresponding to the middle ears which was joined by the fused external auditory canals, Eustachian tubes, and external ears.

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Resumido por el autor, A. G. Pohlman.

Uréteres dobles en embriones humanos y en los del cerdo.

El presente trabajo contiene bosquejos de un embrión humano de 24 mm. de longitud, el cual presenta un doble uréter completo, y del de un cerdo en el cual se presenta la misma estructura; también contiene una serie de dibujos de un cerdo en el cual un uréter aberrante toma la posición excepcional de un uréter inferior situado medialmente respecto al uréter superior. El autor propone una posible explicación de la presencia de esta anomalía del desarrollo, tan poco frecuente.

Translation by Dr José F. Nonidez
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 3

DOUBLE URETERS IN HUMAN AND PIG EMBRYOS

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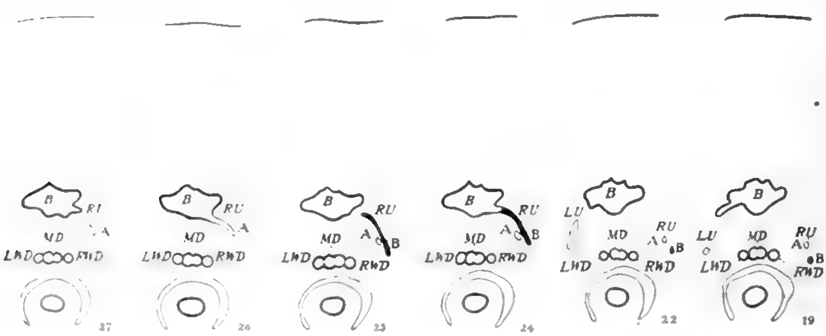
THREE FIGURES

The double ureter has a certain developmental importance in that it furnishes a clew for the disappearance of the cloacal segment of the Wolffian duct, and its manner of incorporation into the bladder. Two cases of complete double ureter were reported in 1905;[1] one is the Mall embryo, no. 175 (13 mm.) and the other in the Keibel embryo, Piper (24 mm.). Both of these embryos show that the ureter from the lower part of the kidney lies dorsal to the ureter from the upper pole, and as they swing around to occupy a lateral position on the Wolffian duct, the dorsal or lower ureter is displaced lateralward, while the ventral or upper ureter lies between it and the Wolffian duct. This rotation from a dorsal position on the Wolffian duct to a lateral one is completed at the time when the cloacal segment of the duct has expanded into the lateral funnel-shaped process of the bladder proper. The accompanying figures, nos. 27 to 24, inclusive, and 22 and 19, are taken from drawings made of the Keibel embryo, which is at present not available for study. Figure 27, the lowest of the series, shows the convexity of the right ureter A, and 26, the section immediately above it, shows the opening of this ureter into the bladder. The next section up indicates the convexity of ureter B, indicated in black, and section 24 its orifice in the bladder. In figure 22, ureter B lies slightly dorsolateral to ureter A, and the convexity of the curve of the left ureter is shown with its orifice in the bladder

[1] Abnormalities in the form of the kidney and ureter dependent on the development of the renal bud. A. G. Pohlman, Johns Hopkins Hospital Bull., vol. 16, Feb., 1905.

three sections higher up (19). This latter figure shows the double ureter on the right lying almost in the sagittal plane, i.e., the ureter B from the lower pole of the kidney practically dorsal to ureter A from the upper pole. Both of these ureters have already migrated to a position cephalad to the opening of the Wolffian duct, and the interval between the orifices is so small that in a gross specimen they might readily be mistaken for a single orifice.

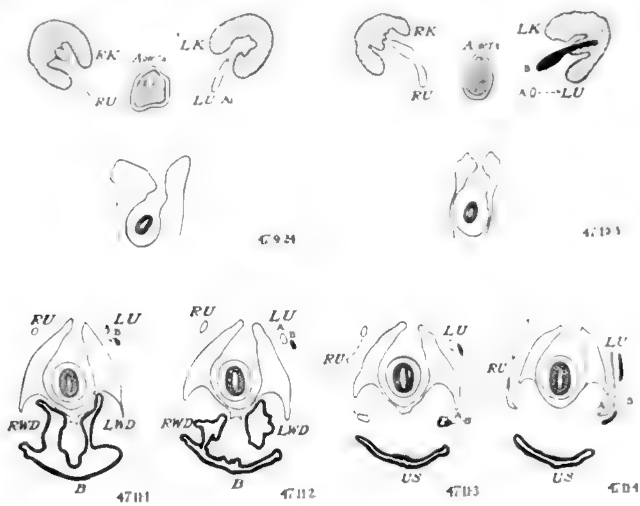
In the Mall embryo the orifices of the two ureters and the Wolffian duct are, so far as I was able to determine them, in common. It would seem, therefore, that where we have wide displacements of the orifices of the two ureters they are to be



found in displacement downward of the median one, i.e., the one lying between the normal ureter orifice and the opening of the Wolffian duct.

I also called attention to the fact that the rotation of the ureter around the Wolffian duct was entirely independent of kidney rotation and position, and that the displacement of the ureter was completed at the time that the cloacal segment of the Wolffian duct was definitely incorporated into the bladder. In the pig the rotation seems to be accomplished before this absorption is completed, so that the ureter opens laterally into the Wolffian duct at some distance from the orifices of the duct into the urogenital sinus.

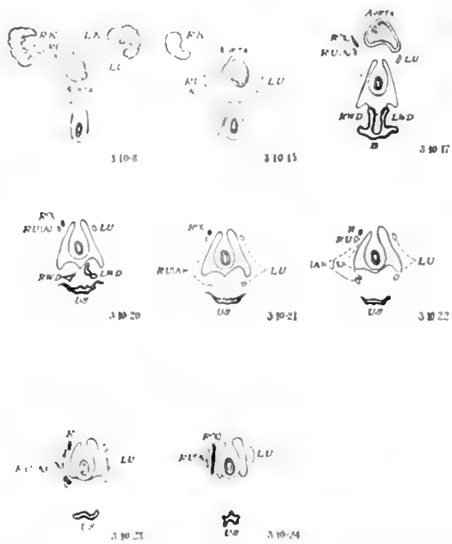
I have found but two pigs with evident ureteral duplication or diverticulae. Pig 47 has a complete double ureter on the left side. Figure 9-24 shows the emergence of the upper ureter from the pelvis of the kidney. Figure 10-5 shows that of the lower ureter from its pelvis. In 11-1 the ureter from the lower pelvis B is already lateral to ureter A from the upper pelvis, and maintains this position through 11-2, 11-3, and 11-4. In 11-3 the two ureters open into the lateral expansion of the Wolffian duct in about a normal position as indicated in the normal ureter on the right; ureter B lying in front of ureter A.



The only essential difference between the relations as shown in fig. 47 and the human embryo is that the twist of the two ureters in the pig appears to run to a higher level than in the human being, and, second, that a cloacal segment of the Wolffian duct persists in the pig at the time that the rotation has been completed.

Cases have been reported in the human being, however, where this general rule, that the ureter from the upper pelvis comes to lie medial to the ureter from the lower pelvis and in consequence has a lower orifice in the bladder, does not obtain. Weigert,

Hyrtl and Kerr[2] have reported cases in which the rule above stated has not prevailed. Kerr has made a report on a case of a double ureter in which the ureter from the upper pelvis lies lateral to the ureter from the lower pelvis as the two curve toward the bladder. In so far as I am aware, there is no developmental explanation for this anomaly, however, a somewhat similar condition was found in pig 3. Figure 10-8 shows the origin of the right ureter from the kidney. Figure 10-15 shows the lower pole of this kidney with the two ureters, right and



left, running forward. In figure 10-17 shows the right ureter A, and behind it a blind ending ureteral diverticulum indicated in black and marked RX. Following this down 10-20 shows it somewhat more medial than in 17, and in 10-23 and 10-24 this lower dorsal ureteral diverticulum comes to occupy the position of the normal ureter from the upper pelvis of the kidney. It appears to end blindly or to fuse with the right ureter in 10-22. Unfortunately, this pig series was cut at 50μ and the finer details of structure cannot be definitely determined.

[2] Complete double ureter in man by A. T. Kerr, Anatomical Record, vol. 5, 1911, p. 55.

It would, however, appear possible that this diverticulum to form a second ureter might have arisen from the first ureter but close to its orifice in the Wolffian duct, and on its medio-inferior aspect, and that it was merely dragged out in the upward migration of the kidney. However, had this aberrant ureter connected functionally with the kidney proper, it would have taken exactly the position described in these cases which appear contrary to the rule, and would also mean that where the ureter from the lower part of the kidney comes to lie medial to the ureter from the upper part of the kidney, that the two orifices into the bladder are close together, in other words, we would not expect a displacement of the medial orifice away from a normal position.

This would, therefore, merely be an exaggeration of an incompletely double ureter, and it would only differ from the latter by reason of the origin of the lower ureter close to the orifice of the upper ureter in the Wolffian duct. It would, of course, be possible to establish this point quite definitely if sufficient number of ureteral duplications could be observed in serial sections. The anomaly is so unusual that the chance of finding intermediate stages, even in the analysis of large numbers of series, is very slight.

I am merely reporting this anomaly because it is so unusual and because it seems to offer some sort of explanation for the few cases of complete ureter duplication which do not follow the rule.

Resumido por el autor, A. G. Pohlman.

Sobre el empleo de un simple método gráfico para anotar las relaciones de los cortes seriados, particularmente útil para la enseñanza de la Embriología.

El presente método gráfico para demostrar las relaciones de una serie de cortes de un embrión es simplemente un modo conveniente de anotar los niveles en los cuales pueden encontrarse determinadas estructuras. Para conseguir este fin se proyectan los cortes que representan un intervalo de 100 micras sobre papel rayado con intervalos de 2 mm. ayudándose de una guia en forma de escalera con el fin de indicar la sección y fila en cada porta-objetos. Esta proyección representa el espesor de los cortes seriados aumentado veinte veces, y la posición de una estructura determinada en un porta-objetos o en un cierto número de ellos puede indicarse por medio de una linea o marca cualquiera. El objeto de este método gráfico es susministrar la información necesaria sobre una serie de cortes de un embrión de un modo más accesible al estudiante y hacer también más accesible para el instructor la corrección de las observaciones. Este método es también aplicable para anotar observaciones en los trabajos de investigación.

Translation by Dr. José F. Nonidez
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 3

THE USE OF A SIMPLE GRAPHIC METHOD OF RECORDING THE RELATIONS IN SERIAL SECTIONS, PARTICULARLY FOR USE IN TEACHING EMBRYOLOGY.

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FOUR CHARTS

Embryology is conceded to be one of the most difficult courses in the medical curriculum, and this may be true for a number of reasons: first, because of the position it occupies in the medical schedule; secondly, because the embryology text-books are descriptive, detailed, and therefore more or less deadly reading; thirdly, because the substance of the course involves a more or less general knowledge of the morphological relations before the student is informed regarding the end-product of the system he is studying; fourthly, because a study of serial sections is imperative and demands the ability of constructing two dimensional pictures into a three-dimensional whole; fifthly, there appears to be a fear in the minds of most teachers that the student will not 'cover the entire subject' in the time allotted to it, and, lastly, many of the teachers of embryology are not sufficiently concerned with the pedagogy involved in so complicated a subject. It may be well to consider some of these topics in the reversed order.

What is true of the pedagogy in embryology, or the lack of it, may hold true of all of the medical subjects and indeed all of the higher branches in learning. The teacher is apt to confuse information with education and, because of his experience in his particular branch and his acquired ability to digest the facts, believe his exposition to be as clear as plate glass. He may come to regard his students as more and more hickory-pated because the same fool questions are asked year after year with

sickening regularity. The student, however, may not appreciate and digest the facts so readily because of his inexperience and because this one particular course is not the only one he is attempting to pigeon-hole in his gray matter. The student may regard these so-called 'self-evident' facts as by no means transparent and may come to look upon his instructor as a sort of human squid elected to make inky the otherwise clear and limpid fountain of knowledge. Embryology is not easy to acquire and the learning is beset with many pitfalls for the unwary. It may perhaps be a trite suggestion that each man look into his methods of teaching with the same zeal shown in his research and see if it is not possible to make the subject more interesting. Let the psychic juice of interest be wanting, and even the most delectable mental pabulum will neither be digested nor assimilated. The processes in embryology, in so far as we understand them, are mysteriously simple, and do we not make a great mistake in our attempts to make tangible that which is quite beyond our comprehension!

The fifth point mentioned is also one in which embryological teaching is not the only sinner. Each man believes his course is important, if not most important, and once given this attitude it is very simple to make the medical student swallow the entire sheaf in order that perchance a few of the grains of truth will stick. The success in imparting the general fundamental principles underlying the development does not include the fatness of the text-book with its dreaded assignments nor the richness in armamentarium in models, series, injected and cleared specimens, and what not. The very accessible facts may be rendered the more inaccessible by placing a halo about them and by saying, "In this way only may the relations be understood." A maximum of material must be investigated in the allotted time, but with a minimum of detail so that the facts themselves may not be made too obscure. Do we overload our students with the number of series? Are the series cut at a maximum rather than at a minimum thickness? Do we require our students to spread over too large a territory and obtain as a result the mere smattering of this, that, and the other system?

Are we afraid that the practical thing is a thing to be avoided in a subject of purely theoretical value, or do we remember that the practicability of the thing is merely a reflection of the theoretical thoroughness? Do we ourselves always know the theoretical and fundamental basis of the things we teach, or do we conveniently refer to the text and say, "It is so written?" It is true that one can make a student study, but one cannot make him think. Spoon-feeding is probably the worst form of instruction, but on the other hand may not the babe, no matter how hungry, go to sleep over his bottle if the contents is made too inaccessible?

The fourth point made was the inability of the student to resolve two dimensional pictures in the series into the three-dimensional whole. This ability seems to be readily developed in some, and in others it is well-nigh impossible to acquire. It is necessary that the student draw sections in order that his information may be accessible to himself as well as to his instructor. Drawing, however, is a sort of reflex from the eye quite comparable to the stenographer's reflex from the ear and does not necessarily imply information. Drawing in some courses is like the busy work in the kindergarten. It helps to rivet the attention of the student on his work and makes for peace in the class-room. It is a good thing and it may also be overdone. To gain the same end-results as accomplished in the alleged artistic efforts of the students and incidentally to delegate the major part of the work above the region of the cerebellum, the following scheme was tried and is presented without prejudice.

The objection to the usual series issued to the student is that they are of too many forms and of far too many sections. I suggest the study of one form, the pig, and leave it to the instructor to use other forms where they show particular points of interest. I would also recommend that the details of maturation, fertilization, cleavage, gastrulation, and the varieties of germ-layer formation be delegated to the preparatory course in the premedic years, preferably in the department of zoology. The course of embryology in the medical school should be largely one of organogenesis.

Two series of pigs should be selected, one of from 9 to 12 mm. and the other from 14 to 18 mm. greatest length. The embryos may be stained in bulk, embedded in celloidin and cut cleared in xylene-cedar oil or xylene-castor oil after the Fish-Gage formula. The larger ones may be cut at 50μ and the smaller ones at 33μ. While sections of this thickness are not to be recommended for research purposes, it is well to employ them for students, because they are too thick for the satisfactory use of an objective of over 16 mm. because the sections may be rescued if a slide is cracked or broken, and because the number of sections is reduced materially.

Carmine, paracarmine, or alum cochineal serves the purpose of a bulk stain; the latter perhaps better than the former two. It is well to have a range of embryo sizes varying from 9 to 18 mm. rather than giving out two series of more or less definite developmental stage. The student provides himself with the usual physics laboratory cross-section paper, 8 x 10½, and ruled 18 cm. by 24 cm. in 2-mm. squares. He takes the two series issued to him and marks the sections at 100 intervals with a dot of ink; that is, every other section at 50μ, and every third one at 33μ. The slides are numbered with a Roman numeral, the rows of sections on the slides indicated by a letter A, B, C, D, etc., and the number of dots in each row by Arabic numerals Thus

Slide I – A – 5
B – 4
C – 5
D – 4

equals 18 dotted sections and the thickness, therefore, of all of these sections, if piled, would equal 1800μ, or 1.8 mm. If each 100μ interval is to be plotted on the graph paper, we must assume a magnification of twenty times. I suggest the use of the stair-case guide shown in the accompanying charts, ruling across for each row lightly, and marking the intervals between the slides with a heavy line. When both embryo series have been transferred into terms of thickness of all sections, times twenty, the student is ready to plot the structures to be observed.

The general idea of this graphic method is very simple. The length, breadth, and the relations of the structures to be shown are entirely eliminated, and all the student does is index the levels at which a given structure is to be found by drawing a line which may be bent into any sort of curve, depending on his particular fancy.

We must remember the line only indicates the level and in structures which are large, and which twist about, only one wall may be indicated. The lines, therefore, in the charts show, for example, the ventral wall of the neural canal, the ventral wall of the descending aorta, the convexity of the upper branchial arches, the concavity of the pulmonary arch, and the point where the lumen of the intestine shows the turning of a corner. If we start with reasonably simple things, like the chorda dorsalis and the ventral border of the neural tube, we establish a sort of string through the entire length of the embryo and demonstrate in what way the tail is bent upon the embryo and upon itself. Once the emergences of the spinal nerves or the extent of the spinal roots are plotted and the position of the tip of the snout shown, the student usually can orient his schematic diagram to correspond with sagittal sections or the pictures of mid-sagittal sections. The method is simple to explain and even much easier to do. I can recommend it heartily as a routine laboratory procedure as well as a method of establishing the position of various structures in the series of embryos of a large collection so that each observer may indicate where the several things which he has studied are to be found. For example, in pig no. 16, 11 mm. the pulmonary artery arises from the last branchial arch in slide no. II, row C, section 4, and the carotid arch is still complete as shown in row A, section 2 of the same slide. The hepatic ducts join the cystic in pig 1, slide III, row A, section 4. This pig is 12 mm. long and shows no tail gut.

By making a master guide for each embryo, any and all sheets of cross-section paper may be used in recording structures, and these observations may be transcribed to the completed graphic representation as indicated in the charts. I have not as yet found two embryos near enough alike to make it possible to confuse one graph with another, and would not hesitate to pick

out of eighty or a hundred unknown graphs the one which would fit the particular embryo slide given me. Changing the embryo number each year avoids all possibility of copying, and the student, once his graphs are made, can readily compare his findings with those of any other student. For example, the carotid arch has disappeared in embryo no. 1, but is to be found in no. 16. One can readily pick out a pig series and the section in the pig where the dorsal limb of the arch is becoming rudimentary. Instead of studying two series, therefore, the student has two series for each man in his class to draw upon for any one point.

I present the accompanying diagrammatic charts, which include all I require of my men, in the hope that this method may prove satisfactory and will make for the elimination of a large amount of the busy-work which detracts from the interest in a course of embryology.

Under Ecto

2, optic cup or eyeball
LD, lacrimal duct
J, Jacobson's organ
5, ganglion of trigeminal nerve
7, ganglion of facial nerve
IE, membranous labyrinth
H, hypophysis
N, ventral border of neural canal
T, first thoracic spinal ganglion
L, first lumbar spinal ganglion

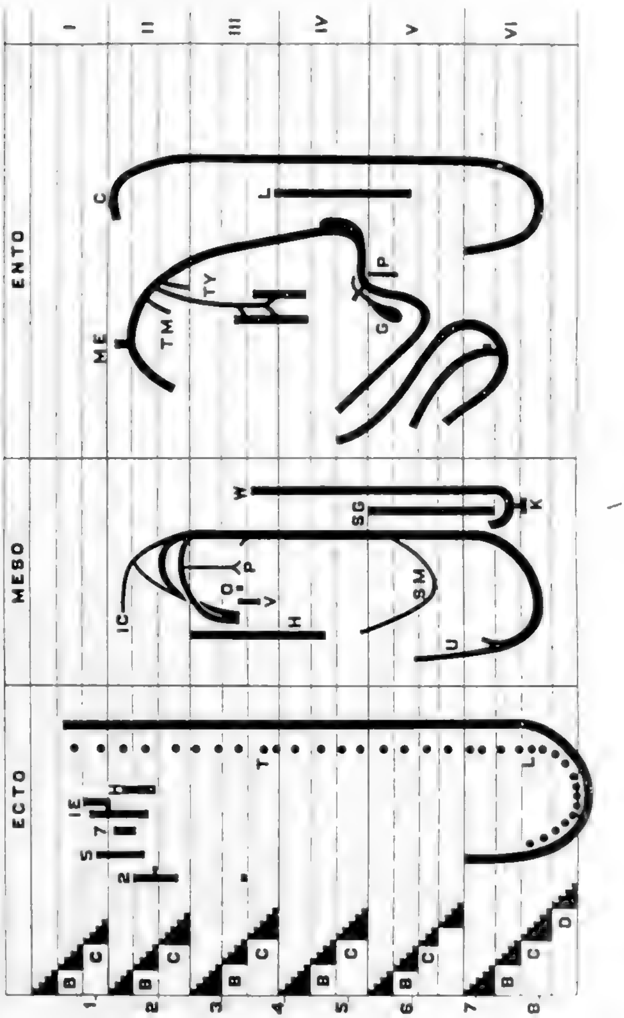
Under Meso

IC, internal carotid artery
EC, external carotid artery
RS, right subclavian artery
LS, left subclavian artery
H, extent of heart
O, foramen ovale
V, ventricular defect
P, origin of right and left pulmonary arteries
SM, superior mesenteric artery
U, umbilical artery
I, II, III, first, second, and third branchial cartilages
SP, spleen
A, adrenal
SG, sex gland
M, Muellerian duct
W, Wolffian body and duct
K, kidney and ureter

Under Ento

ME, middle-ear diverticulum
TM, thymus
TY, thyreoid
T, trachea
C, chorda dorsalis
L, liver
G, gall bladder
P, pancreas
B, urinary bladder
UG, urogenital sinus

The Roman numerals indicate the slide number and the Arabic numrals millimeter intervals or finally the length of the pig computed by multiplying the number of sections in the series by the section thickness.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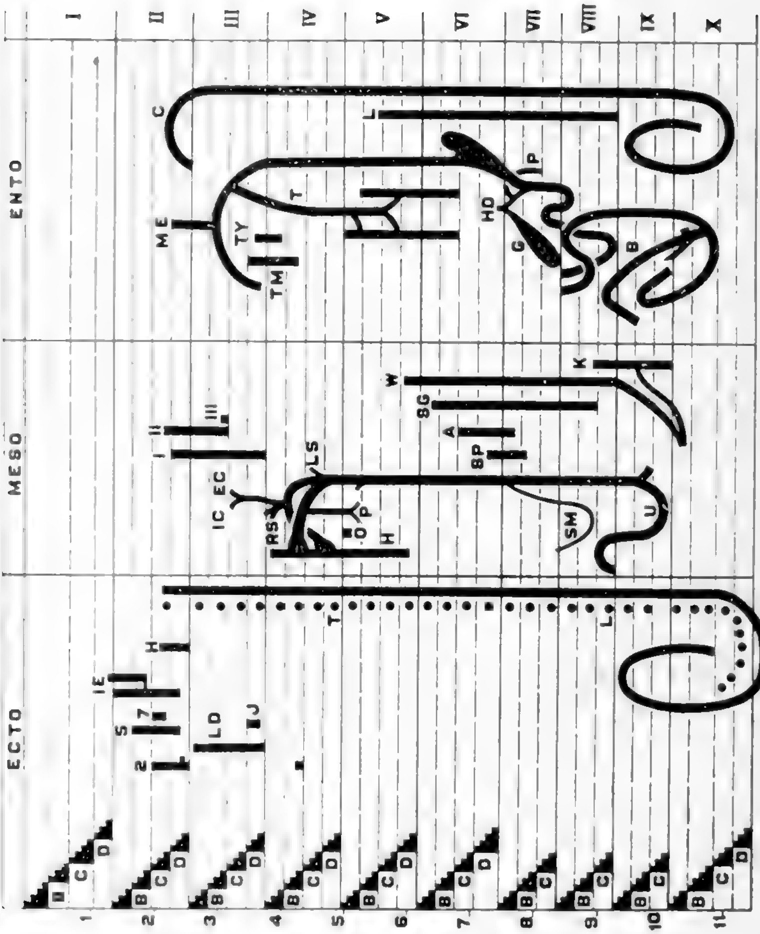

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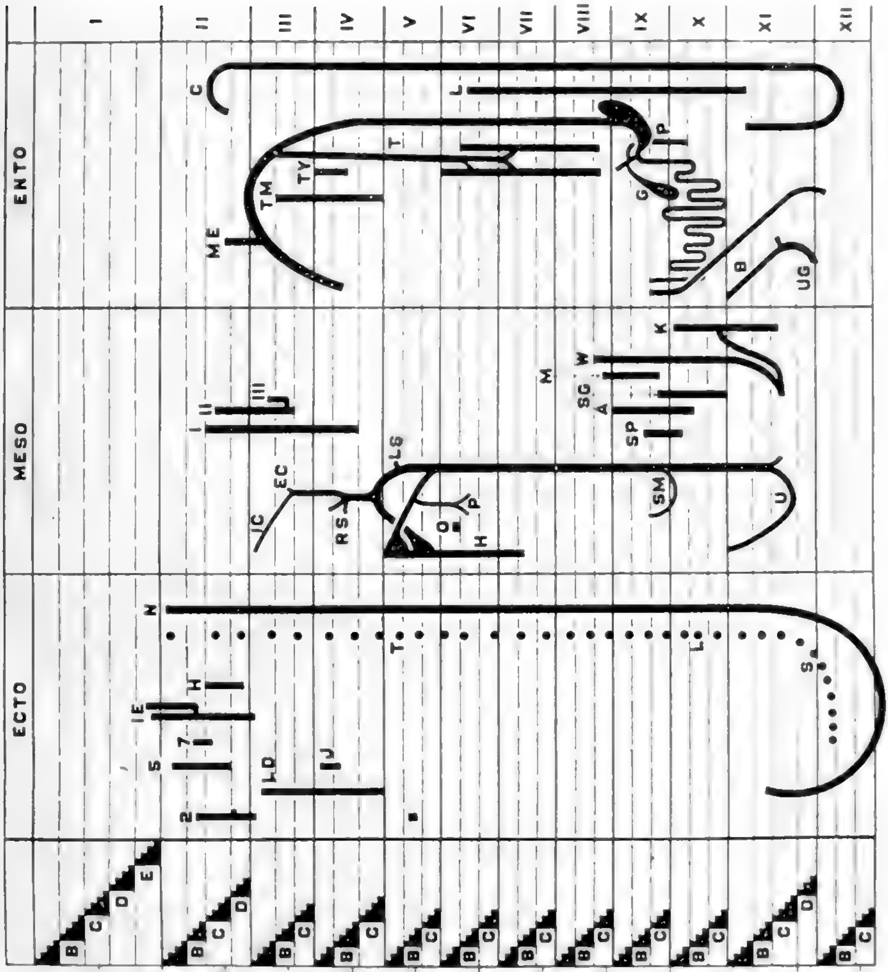
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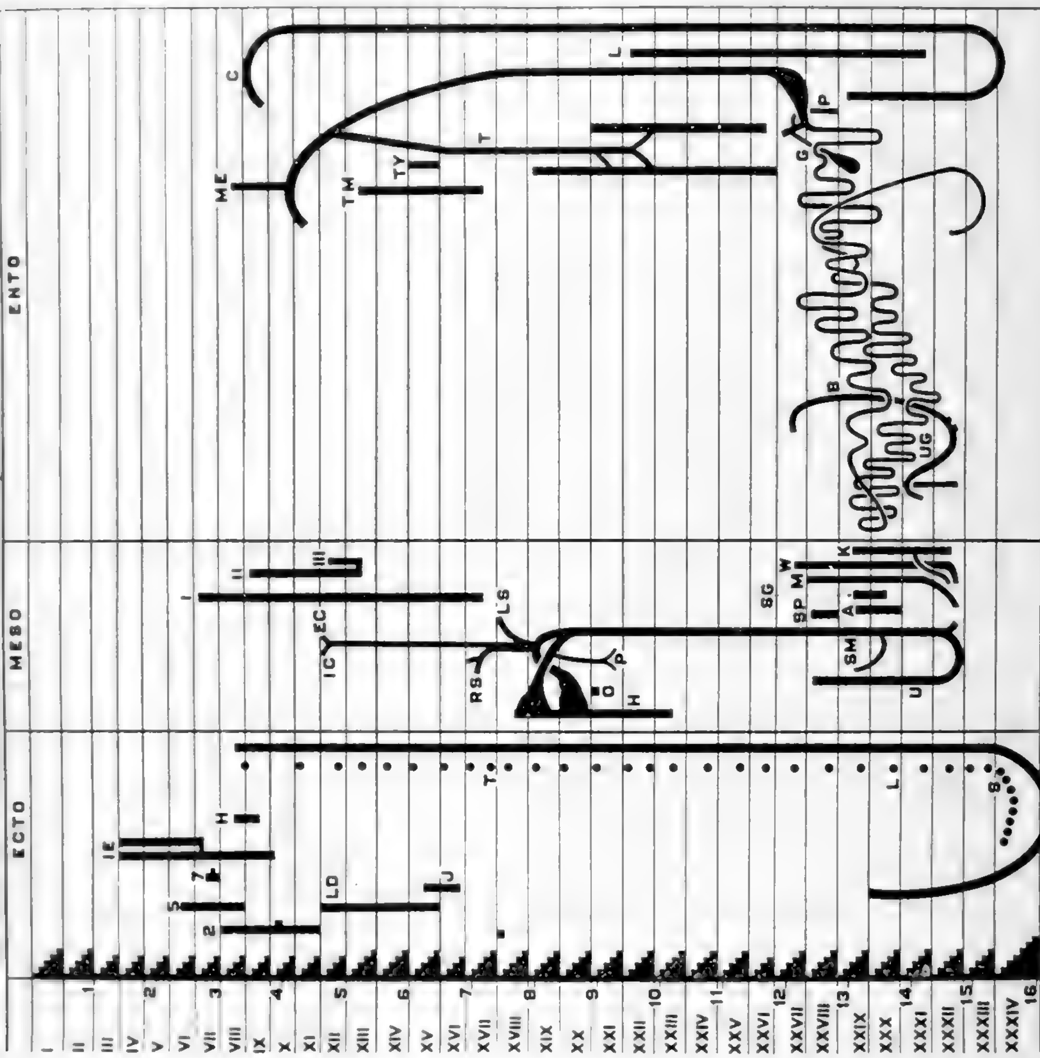
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D

Resumido por el autor, A. G. Pohlman.

Sobre el empleo de la cera de bayberry (1) para endurecer los bloques de parafina.

La cera de bayberry de un punto de fusión de 45° a 49°C. se funde y filtra. Cuando se agrega en proporción de 10 por ciento a la parafina de un punto de fusión de 52°C. se obtiene un bloque que presenta los mismos caracteres favorables para obtener cortes que la parafina dura de 61° a 62°C., con la ventaja de rebajar en 10° el punto de fusión de la misma; un 15 a 20 por ciento de la mencionada cera produce una mezcla de un punto de fusión tan bajo como 51° a 52°C., la cual presenta mayor dureza que la parafina más dura sin la desventaja de la textura cristalina que presenta esta última. El presente método se propone para cortar secciones de diverso espesor a las temperaturas extremadamente variables del laboratorio, ajustando el caracter del bloque a dicha temperatura en vez de hacer lo contrario.

Translation by Dr. José F. Nonidez
Columbia University

(1) Myrica cerifera.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 3

THE USE OF BAYBERRY WAX IN HARDENING PARAFFIN BLOCKS

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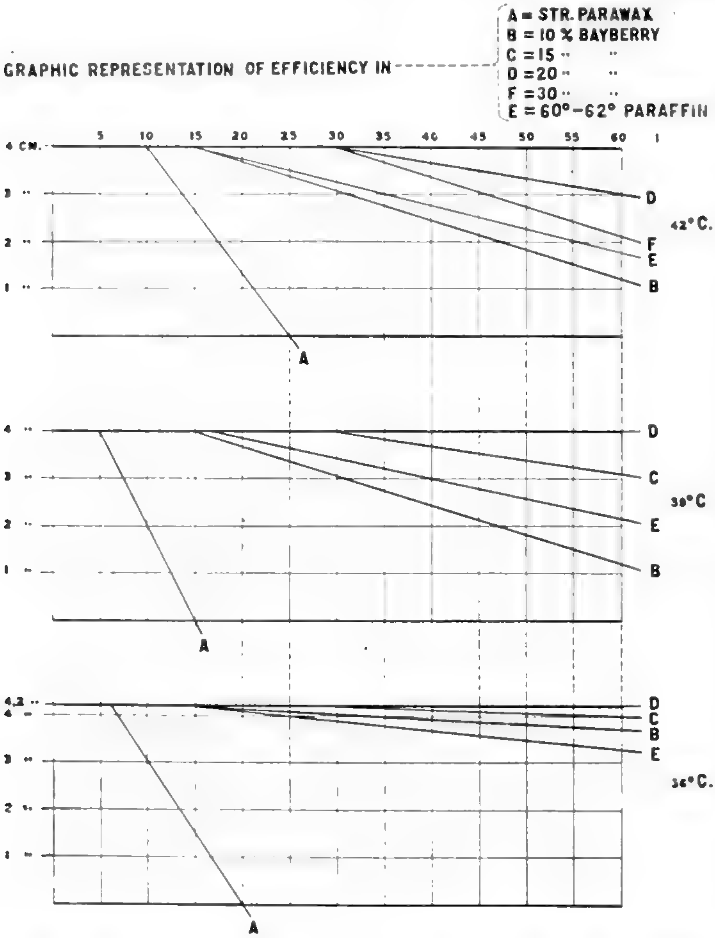
ONE CHART

It is well known that paraffin enjoys a limited range of usefulness, particularly where temperature fluctuations are marked, especially in extremely warm weather. Soft paraffins 48° to 50° are therefore only available for thick sections at low room temperatures; hard paraffins 60° to 62° develop a crystalline texture at the expense of the gummy property, and not only have the undesirable feature of requiring a high embedding heat, but are extremely sensitive to small fluctuations in ordinary room temperatures. We may mix hard and soft paraffins with a resultant of a melting point of about 53° to 55°, gaining good qualities from both, or may procure, already mixed, parawax of about this melting point at low cost. Parawax has proved a desirable material for embedding, and to this end the writer investigated the possibility of hardening parawax or soft paraffin without increasing the melting-point. The following experiments were conducted with bayberry wax or tallow:

The material is well known and inexpensive. It comes in light brown lumps with a slightly greenish cast (probably chlorophyl) and with a somewhat floury surface. It is brittle, and may be ground to a powder, if finely flaked, at ordinary room temperatures. Though somewhat variable, its melting-point is about 45° to 50°C. The wax was heated and filtered. It was found to have a melting-point of 49° C.

This material was added to parawax of determined melting-point, 52° to 53°C. in proportions of 10 per cent, 20 per cent and 30 per cent, and blocks constructed of pure parawax (A); 10

per cent bayberry (B); 20 per cent bayberry (D); 30 per cent bayberry (F); and hard paraffin (E), tested melting-point 61°C. The blocks, 9 x 22 x 40 mm., were affixed to a slide by a small end so that when the slide was placed in a box the long axis of



each block was horizontal. All of these blocks were introduced into an electric incubator set at 42°C. and watched for an hour to observe the 'wilting.' The figures at the top of chart 1 represent time; while the figures at the right show the height of the block, i.e., the amount of bending. The parawax began to

bend in ten minutes 10 per cent bayberry at fifteen minutes, hard paraffin at fifteen minutes, and both 20 per cent and 30 per cent bayberry in thirty minutes. At the end of twenty-five minutes the parawax block had completely turned down and the height of the blocks at the end of the hour is indicated at the right. This showed that while 10 per cent and 20 per cent bayberry had a marked hardening, 30 per cent was beyond the optimum, and the tests were repeated at 38°, using 15 per cent instead of 30 per cent.

Here a wire nail 6 cm. long was melted into the center of each block so that the nail stood horizontal and the heights at the right show the amount of wilting in terms of displacement of the nail head. The blocks were 9 x 9 x 24 mm. Parawax wilted in five minutes, 10 per cent bayberry in fifteen minutes, hard paraffin in seventeen minutes, 15 per cent bayberry in thirty minutes, and 20 per cent bayberry not at all.

The same was again repeated at temperature of 36°C., using blocks 9 x 9 x 9 mm. Parawax wilted in seven minutes, hard paraffin and 10 per cent bayberry at fifteen minutes, 15 per cent bayberry at thirty minutes, and 20 per cent bayberry not at all.

The melting-points of these mixtures, 10 per cent, 15 per cent and 20 per cent, is slightly lower than the parawax or 50° to 51° —10 per cent addition created a block which is as hard as hard paraffin 61° with 10° less melting-point,—while 15 per cent and 20 per cent harden the parawax beyond any hard paraffin obtainable.

The bayberry tallow added in small amounts, say 5 per cent or 10 per cent to hard paraffin, not only hardens it, but eliminates certain undesirable cracking and crystallization in that material. The wax is about as soluble in xylene or toluene as paraffin and far more soluble in chloroform. The disadvantages in using the bayberry is to be found in lowered transparency and a slight brown color. The material cuts just as well and even better than hard paraffin.

In using bayberry tallow caution must be observed in letting it harden in a pure state in a beaker because it will break the bottom out of it. Again, if thought advisable, the chlorophyl

may be dissolved out by adding 100 grams wax to 250 cc. 96 per cent alcohol; bring the alcohol to a boil and cool off gradually. The wax will precipitate out in the form of globules of darker brown and a flocculent light precipitate. Filtration and driving off alcohol on a water-bath yields a light brown material with only a trace of green in it. The globular precipitate has a slightly lower melting-point than the flocculent one, but I do not consider the alcohol treatment or the separation of these two elements desirable or necessary.

I suggest the use of this method for hot-weather work or in extreme hot weather to add 10 per cent, 15 per cent, or 20 per cent to bayberry wax hard paraffin to increase the efficiency of the block and to reduce the melting-point one or two degrees.

Ether embedding technique as suggested by Federici for paraffin (Encyclopedia D. Mikro. Technik, 2. Auflage, Bd. 2, S. 361) may also be satisfactorily done with bayberry wax.

Resumido por el autor, A. G. Pohlman.

Una modificación de la placa de cera y papel empleada en el método de reconstrucción de Born.

Esta placa es una lámina de cera obtenida comprimiendo cera caliente por medio de un rodillo, la cual lleva pegado papel de dibujo a una de sus caras. El calco del dibujo, obtenido mediante papel carbón, se coloca con la superficie que lleva el dibujo sobre la superficie parafinada y comprimiendo por medio de un rodillo se obtiene el dibujo sobre ella; se vierte cera fundida sobre el dibujo hasta la altura que marcan las guias y se nivela con un rodillo caliente. La parafina entre el papel y la superficie de la placa permanece fundida cuando la cera está bastante endurecida para retirar la placa obtenida. Esta método combina ciertas ventajas de la placa de Born y del de la placa obtenida vertiendo cera y remedia algunas de las desventajas de ambos.

Translation by Dr. José F. Nonidez
Columbia University

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 3

A MODIFICATION OF THE BORN PAPER-WAX RECONSTRUCTION PLATE

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The Born method of applying the third dimension to serial drawings for purposes of reconstruction has many advantages over the blotting-paper and 'poured' wax plate. All three methods work well in the hands of experienced men. The great disadvantage of blotting-paper is that it cannot be used for thick plates, i.e., over 1 mm., while under 1 mm. thicknesses are readily handled, especially if impregnated with hard paraffin, when they may be cut quite readily with a sharp knife on a glass plate. The poured plate of 1 mm. in thickness is too friable and is likely to undergo distortion in the cutting and piling. Hence it is used practically only in 2 mm. thicknesses. The Born method of applying the wax to the carbon copy of the serial drawings has many advantages, especially if made on specially devised machines, such as that of Huber at Ann Arbor or my own at Johns Hopkins. The latter is calibrated to make accurate plates at twentieths of millimeters, and has the great advantage of making possible resulting models of uniform size no matter what the section thickness and magnification. The Born plate is briefly made as follows: The carbon copy of the serial drawing is squeegeed to the surface of the stone or metal slab with turpentine. Melted wax, preferably wax paraffin resin, is poured over the drawing to a thickness slighly exceeding that of the strips or guides and leveled with the hot roller. As soon as the surface of the wax is firm, it is brushed lightly with turpentine and a sheet of tissue-paper applied and again rolled. The plate is trimmed and placed between blotters to dry. The great disadvantage in this method is the use of turpentine which is very disagreeable, particularly when hot, and

which makes the wax sticky. Further, it tends to dissolve off the carbon copy, takes a long time to dry, and, finally, does not incorporate either the drawing or the tissue-paper into the wax, so that they tend to peel off in the cutting. The tissue-paper backing also makes it more difficult to fuse the plates successfully in building the model.

The modification suggested is the following: Place the carbon copy of the drawing face down on the stone slab. Pour a few teaspoonfuls of melted paraffin on the paper and squeegee the paper to the slab with the hot roller, passing it at right angles to the guides. Scrape the upper surface of the paper with a broad painter's knife, to get rid of the excess of paraffin and avoid blisters. Pour on the wax and level with the hot roller as before. As soon as the plate is firm, trim to the edges of the paper and lift off. The pure paraffin between the paper and the slab is still melted when the wax is quite firm. Place the plate on some smooth surface, cover the wax surface with paper and put a board on it to overcome the curling. The plate is ready for use as soon as it is cold.

The advantages of this method are as follows: First, the turpentine is entirely eliminated and, second, the plate is not backed with tissue-paper. It compromises the poured and the rolled plate with all of the advantages of both and with none of the disadvantages of either. The carbon copy is fixed with the paraffin and the paper is so thoroughly incorporated into the wax that it may only be torn off bit by bit. The plate is cut, paper side up, and gives a sharp edge without the thickening found in the poured plate.

Resumido por el autor, Harvey Ernest Jordan.

La histogénesis de las plaquetas sanguíneas en el saco vitelino del embrión del cerdo.

Mediante el empleo de la técnica de Wright se puede demostrar la presencia de plaquetas sanguíneas típicas en los sinusoides del saco vitelino del embrión de cerdo de 12 mm. Estas plaquetas se forman principalmente a expensas de células gigantes y también, en cierto grado, a expensas de los linfocitos primitivos o "hemoblastos" y a veces se derivan de las células endoteliales. Estas últimas deben interpretarse como hemoblastos que se están diferenciando del endotelio. Las células gigantes son, en esencia, hemoblastos hipertrofiados. Tanto los hemoblastos como las células gigantes que de ellos derivan se caracterizan por la presencia de gránulos metacromáticos en su citoplasma. Las plaquetas se originan de dos maneras diferentes: por segmentación de pseudópodos y por fragmentación de áreas citoplásmicas de mayor tamaño. Una de estas maneras está asociada con una función aparentemente normal, la otra con procesos degenerativos, como indica la condición anormal del núcleo. Tanto los hemoblastos como las células gigantes pueden diferenciarse en eritrocitos. Las células gigantes hemogénicas del saco vitelino están representadas en la médula roja de los huesos por elementos homólogos. Los osteoclastos no contienen gránulos metacromáticos; su ausencia susministra un criterio exacto para diferenciar las células gigantes hemogénicas de las osteolíticas, en la médula ósea. La formación de las plaquetas es un proceso idéntico en el saco vitelino y en la médula roja de los huesos. Las plaquetas aparecen como un producto accesorio de la actividad normal de los leucocitos con gránulos metacromáticos, la cual se manifiesta por la formación de pseudópodos, y también como resultado de procesos degenerativos, que se manifiestan por cambios nucleares acompañados de una fragmentación del citoplasma granular metacromático.

Translation by Dr. José F. Nonidez
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 3

THE HISTOGENESIS OF BLOOD-PLATELETS IN THE YOLK-SAC OF THE PIG EMBRYO

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INTRODUCTION

In his article on the origin of blood-platelets from megakaryocytes in the bone-marrow of certain mammals, Wright[10] describes and figures also certain megakaryocyte 'forerunners' in the blood of young guinea-pig embryos. In this paper also he offers the hypothesis that the amphibian homologue of the mammalian megakaryocyte is the spindle cell. The fundamental question here involved concerns the significance of the blood-platelets. The present investigation aims to further elucidate this problem through an approach by way of the giant-cells of the yolk-sac of the pig embryo.

As regards the embryonic 'forerunners' of the megakaryocytes, Wright states that blood-platelets are present only after these cells have made their appearance, in guinea-pig embryos of about 4.5 mm. length. After this stage of development the 'forerunners' of the megakaryocytes occur free in the blood-vessels; they then have a size about that of the erythrocytes, and contain the characteristic metachromatic (red to purple) granulation of the larger megakaryocytes. Both the smaller 'forerunners' and the transition forms are said to break up in the blood-vessels into typical blood-platelets just as do the fully developed megakaryocytes. Certain of these 'forerunners' are described as originating from the endothelium of the blood-vessels, and one such progenitor is figured still in connection with the endothelium, but containing the cytoplasmic granules characteristic of megakaryocytes. These 'forerunner' cells

would seem to call for further study. A more complete interpretation is made possible on the basis of the data given below as derived from a study of the yolk-sac of the pig.

It seems desirable at this point to recall the scope of Wright's work, and to emphasize the cogency of his arguments, based upon data which amount to a practically complete demonstration, that blood-platelets arise by a process of segmentation of the pseudopods of megakaryocytes at certain stages of their development. Wright studied the red bone-marrow and spleen of the cat, kitten, man, mouse, dog, rabbit, guinea-pig, white rat, and opossum. The results of the study of this variety of material consistently support the same conclusion. This conclusion was confirmed by the work of Bunting[1] and that of Downey[4] for the rabbit. Ogata[11] also has confirmed Wright's conclusion in every respect (cited from Downey). A careful study of the red bone-marrow of the rabbit and of the guinea-pig, treated according to Wright's technic, has convinced me also of the accuracy of Wright's conclusion regarding the giant-cell origin of the blood-platelets.

Wright's hypothesis of the homology between the thrombocytes of ichthyopsid and sauropsid bloods and the hemogenic giant-cells of mammalian hemopoietic organs is based chiefly on his observation with regard to the spindle cells of the blood of Batrachoceps attenuatus, where also the cytoplasm contains metachromatic granules and where portions are regularly pinched off to form corpuscles structurally and tinctorially very like mammalian blood-platelets. Downey[3] describes similar 'azurophil' granules in the spindle cells of Amblystoma, but fails to find cytoplasmic constrictions; he nevertheless believes that Wright's conclusion 'that the spindle cells correspond to circulatory megakaryocytes is justified' (p. 313). I have seen this same phenomenon of pseudopod fragmentation also in the case of the thrombocytes of the blood of the frog, Rana pipiens. An attempt will be made to formulate inclusive and consistent reinterpretations of this body of data in the light of evidence derived from the study of the yolk-sac of the pig, combined with certain observations regarding the primary lymphocytes (hemo-

blasts) of the marrow of the frog. The latter will be discussed more in detail in a separate paper.

Attention should here be directed also to the differences in details, as revealed particularly by the illustrations, in the process of platelet origin from megakaryocytes as described in the papers of Wright[14] and of Downey.[3] Wright views and illustrates the process chiefly in terms of a segmentation of pseudopods of apparently healthy cells at a certain stage of their development (Wright's fig. 14); Downey, on the contrary, figures the process as one of disintegrating cells, as indicated by their complexly lobulated, wrinkled, non-granular nuclei (Downey's fig. 15). This difference in detail is actually of much importance and demands an explanation. In my study of the red marrows of the guinea-pig and the rabbit I find that both processes (segmentation and fragmentation) occur abundantly. They are quite different in nature, but lead to practically identical results. The matter will be further discussed below.

Still other essential points in this connection concern: 1) The genetic, morphologic, and tinctorial dissimilarity between the osteolytic and hemogenic giant-cells of hemopoietic foci; that is, the osteoclasts and the giant hemoblasts, respectively, the essential phagocytic nature of the former, and the erythroblastic significance of the latter (Jordan[10]). Contrary to the conclusion of Dickson,[2] who identifies all types of giant-cells and ascribes to them in common a phagocytic function, the evidence indicates that the so-called megakaryocytes are not primarily and generally phagocytic. 2) The demonstration that the genetic history of the giant-cells of the yolk-sac traces back to hemoblasts, which may in some cases be traced to the endothelium, and the further demonstration that certain mononucleated giant-cells (genuine megakaryocytes) or large hemoblasts become polymorphonucleated and subsequently multinucleated, in which phase they may under certain conditions become transformed into erythrocytes (Jordan[10]).

MATERIAL AND METHODS

Portions from the proximal pole of the yolk-sac of the pig embryo of about 12 mm. length constitute the chief body of material for this investigation. The tissue was fixed for twenty-four hours in a mixture of 10 parts of formalin to 100 parts of a saturated normal-salt solution of $HgCl_2$, as recommended by Downey. It was then passed through several changes of 70 per cent alcohol, treated with tincture of iodine, and embedded in paraffin. Sections cut at 5μ were stained on the slide according to the technic employed by Wright. Similar tissue fixed in Helly's fluid and stained with eosin-azure, or hematoxylin and eosin, was used for comparison. The several marrows (femurs of frog, guinea-pig, and rabbit) employed for comparison with yolk-sac, certain observations from which enter into the present discussion and contributed to the interpretations here arrived at, were also preserved and stained according to Wright's technic.

DESCRIPTION

We are interested at this time only in the hemopoietic tissue of this yolk-sac, namely, the middle mesenchymal layer ('angioblast') with its network of blood-vessels containing cells at all stages of differentiation from original hemoblasts or even endothelial cells to erythrocytes (erythroblasts and normoblasts). Hemopoiesis is still very active at this stage of development in the proximal portion of the yolk-sac. Giant-cells and platelets are very abundant. The metachromatic (red to purple) granules of these and other cells stand forth with remarkable clearness in this tissue treated with Wright's technic. Comparative studies of the various types of cells in yolk-sacs of the same age prepared by the Helly and Wright technics, respectively, were very helpful in interpreting especially the giant-cells.

In my previous studies of the yolk-sacs of pig[7] and mongoose[8] embryos, I arrived at the conclusions: 1) that certain hemoblasts differentiate directly from endothelium; 2) that the giant-cells are enlarged hemoblasts; 3) that the polynucleated giant-cells are derived from the mononucleated forms, chiefly by

nuclear amitosis leading through polymorphonucleated forms, and 4) that the polynucleated types may produce erythrocytes by a process of intracellular differentiation. This endogenous erythrocytogenesis is practically limited to binucleated forms of giant-cells, though it may occasionally be seen in cells with four nuclei. Such binucleated types occasionally differentiate into structures corresponding to an endothelial cell enclosing an erythroblast. The polynucleated giant-cells are accordingly multiple hemoblasts comparable to blood-islands, an interpretation consistent with the facts of their origin and their function. In a subsequent work (Jordan[10]) it was shown that the hemogenic giant-cells of red marrow, as distinct from the invariably multinucleated osteolytic giant-cells or osteoclasts, have a like origin from hemoblasts, and may under certain conditions apparently function as sources of erythrocyte formation. Only the polynucleated forms apparently differentiate into erythrocytes; transition stages between the hemoblasts and the multinucleated giant-cells are polymorphonucleated forms like the so-called megakaryocytes, and are apparently identical with the latter. Mononucleated, polymorphonucleated and the polynucleated types of hemogenic giant-cells have in common a fundamentally homogeneous and basophilic cytoplasm and contain fine spheroidal metachromatic granules. The three types may give rise to blood-platelets by segmentation of pseudopods or by fragmentation of larger peripheral portions of their cytoplasm.

In the yolk-sac of the pig embryo several forms of giant-cells occur, which after staining according to Wright's technic show structural and tinctorial features identical with those of the corresponding cells of the marrow. These cells likewise produce platelets of varying sizes both by a segmentation of pseudopods and by a fragmentation of their cytoplasm. The former cells are characterized by more normal nuclear features than the latter. There are in addition to these cells still others with a similar cytoplasm and metachromatic granulation: 1) Certain endothelial cells differentiating into hemoblasts and just separating from the endothelial wall, and 2) hemoblasts.

There are apparently certain exceptions to the more typical granulated types among the free young hemoblasts. Such have a deeply blue staining homogeneous cytoplasm. The nucleus is of the same vesicular type, with delicate reticulum, a plasmosome and several net-knots, as that of the hemoblasts with metachromatic granules. Careful examination will in many cases reveal a few metachromatic granules in the cytoplasm of such apparently non-granular hemoblasts. Moreover, the granules first appear about the attraction sphere, thus in a restricted region, and sections may obviously pass through a plane of the hemoblast at right angles to the plane passing through this initial mass of granules. The granulation may make its appearance at variable stages of development, sometimes earlier, even while the differentiating hemoblast is still in connection with the endothelium, sometimes relatively late in the hemoblast stage. In the case of the marrow of the guinea-pig and the rabbit, the beginnings of the granulation can be traced likewise, but the granules apparently first appear in relatively later stages of hemoblast development. As the metachromatic granules increase in amount they scatter through wider areas of the cytoplasm, and the orignally deeply blue-staining cytoplasm changes to a pale blue color. A non-granular hyaline border of variable width can almost invariably be distinguished peripherally in these granular hemoblasts and giant-cells of the yolk-sac. The coincident decrease in the basophily of the cytoplasmic substratum with the appearance and increase of the azurophil granulation suggests a genetic relationship, but the actual steps in the origin of the granules from out of the cytoplasm cannot be discerned.

As the hemoblasts differentiate into erythroblasts, the metachromatic granules disappear. Several granules may occasionally still be seen in the later phases of erythroblast differentiation, scattered in the hemoglobin-containing cytoplasm. In the Helly-fixed tissue the young erythroblast ('megaloblast,' Maximow) has a granular cytoplasm; the hemoglobin seems to originate as initial granules, for this granulation does not occur in the hemoblast forerunner nor in the erythroblast derivative.

Since such granulation is absent from the cytoplasm of the hemoblasts in Helly-fixed tissues, it cannot be identical with the metachromatic granules of the hemoblasts in tissues treated according to Wright's method. This fact contravenes any suggestion that the hemoglobin of the erythroblasts has its direct origin in the metachromatic granules of the hemoblasts. However, the ability of cytoplasm to produce metachromatic granules and hemoglobin undoubtedly resides in the same cell and to some extent at least coincidentaly. Binucleated giant-cells in process of endogenous erythrocyte formation undergo similar perinuclear cytoplasmic alterations in the elaboration of hemoglobin, with a coincident development of a limiting membrane.

The process of platelet formation from the giant-cells and hemoblasts of the yolk-sac presents nothing essentially new. It is exactly like that in the red bone-marrow as regards the megakaryocytes. The process is twofold, that is, either by segmentation of pseuodpods or by disintegration of large peripheral masses of cytoplasm. The resulting platelets are identical and vary much in size. The nuclear characteristics associated with this twofold process are relatively specific. Cytoplasmic disintegration is associated with a peculiar type of nucleus. This nucleus is very extensively lobulated; the lobules are small with a wrinkled contour; certain lobules are very pale, while others are pycnotic; the lobules have a non-granular vesicular character and present a sort of cloudy appearance; net-knots are practically lacking and the nuclear network is either very faint or entirely lost. On the contrary, the nuclei of the giant-cells producing platelets by segmentation of pseudopods consist of relatively fewer and much larger lobules. The relatively robust nuclear wall has a sharp contour. The clear vesicular lobules have a distinct chromatic network with many larger and smaller karyosomes and an occasional plasmosome. The nucleus as a whole has a distinctly healthy appearance in comparison with that of the giant-cells contributing platelets by cytoplasmic fragmentation.

The endothelial cells with metachromatic granules apparently produce platelets only by terminal constriction of short pseudopods. None were seen with long pseudopods, nor were any endothelial cells seen in process of fragmentation. This cell is identical with that described by Wright[14] for the blood-vessels of the guinea-pig embryo.

Certain hemoblasts also are covered with psuedopods. These can be seen to segment off typical blood-platelets. Other hemoblasts suffer cytoplasmic disintegration, producing thus groups of typical platelets and leaving naked nuclei. These cells are identical with those described by Wright for the guinea-pig as 'forerunners' of megakaryocytes. Hemoblasts in all respects like these platelet ancestors differentiate into erythrocytes.

The giant-cells of the yolk-sac are essentially enlarged hemoblasts. As such they may contain a single large spheroidal or reniform nucleus, a bilobed or polylobular nucleus suggestive of the 'megakaryocyte' nucleus of red marrow, or they may be multinucleated. The cytoplasm is identical with that of the giant-cells of the marrow, consisting of a light-blue staining substratum with metachromatic lilac-colored granules. Like the corresponding cells of the marrow, the yolk-sac giant-cells produce blood-platelets by segmentation of pseudopods and by fragmentation of larger cytoplasmic areas. The later steps of this latter process leave a naked nucleus.

This material shows also occasional giant-cells among the entodermal cells lining the yolk-sac. They can be readily identified by reason of the metachromatic granules of the cytoplasm, which causes them to contrast sharply with the entodermal cells with their proximal content of basophilous substance and ergastoplasmic filaments. Spee[13] reported similar polynuclear giant-cells among the entodermal cells of the human yolk-sac and described them as arising from the entoderm. He, moreover, interpreted them as progenitors of red cells. Saxer[12] likewise interpreted similar giant-cells in the yolk-sacs of pig, sheep, and cat embryos as ancestors of normoblasts. In my studies of the yolk-sacs of a 9-mm. and a 13-mm. human embryo[6] I failed to find giant-cells among the entodermal cells; only a few were

seen extravascularly within the mesenchymal layer. Possibly the staining technic employed did not clearly reveal such cells that might have been present among the entodermal cells in these sections. However, the evidence is complete that these giant-cells do not originate from entodermal cells as claimed by Spee, but that they may occasionally wander into this layer from the underlying mesenchyma. But it is of much interest that both Spee and Saxer also interpreted these cells as ancestors of erythrocytes.

Conditions identical with those of the yolk-sac appear also in the liver sinusoids of this stage of development. Certain endothelial cells elaborate metachromatic granules, round up into typical hemoblasts, and separate from the endothelial wall as free cells. Such may produce platelets either during their origin from endothelium or subsequently. Free hemoblasts and giant-cells of the liver likewise produce platelets abundantly.

The liver contains also the peculiar elements, previously described for the yolk-sacs and the intra-embryonic blood-vessels of certain mammals, namely, structures that appear like a cross-section of a capillary containing an erythrocyte, the wall of the capillary being formed by a single endothelial cell with its nucleus at the level of the section. Such structures are interpreted as originally binucleated hemoblasts in which one nucleus with its enveloping cytoplasm has differentiated into an erythroblast, the other into an endothelial cell. The presence of such cells in both the yolk-sac and liver vessels of this stage gives additional support to this interpretation.

The pig embryo of this stage shows also numerous large cell-clusters along the ventral portion of the abdominal aorta. These have been previously interpreted as clusters of hemoblasts differentiating from endothelium which has been invaginated locally into the lumen of the vessels, in some cases at least in consequence of a shrinkage of underlying mesenchyma, due to atrophy of a ventral segmental ramus.[8] The Wright's staining technic reveals metachromatic granules in the cytoplasm of these cells. Thus, on the basis of still another feature is indicated the classification of the constituent cells of these clusters as differentiating erythroblasts.

DISCUSSION AND CONCLUSIONS

The foregoing description of conditions in the yolk-sac of the pig embryo indicates the complete correspondence of the cells with metachromatic granules (endothelial cell derivatives, primitive free lymphocytes or hemoblasts, and hemogenic giant-cells) with similar cells in the blood-vessels of guinea-pig embryos and the red bone-marrow of adult mammals as first described by Wright.[4] It becomes evident also that similar cells occur in the liver sinusoids, and that the aortic cell clusters consist of elements corresponding with the hemoblasts of the yolk-sac. The evidence shows also that typical blood-platelets may originate from any cell with metachromatic granules. It shows, further, that all types of cells with these granules are derivatives of a common lymphocyte-like cell or 'hemoblast.' At least a certain number of the latter differentiate from endothelium. Blood-platelets occur abundantly in the blood-vessels of the yolk-sac of the 12-mm. pig embryo. Since they arise from hemoblasts at all stages (except possibly the very earliest), in some cases before the cell has separated from the contributing endothelium, the conclusion seems justified that a certain few are present almost coincidentally with the appearance of the first blood-cell progenitors (primitive lymphocytes or hemoblasts). It does not seem probable, therefore, that platelets first arise at about the 4.5-mm. stage in the guinea-pig embryo as stated by Wright. An examination of the yolk-sacs of earlier stages might reveal platelets in abundance.

In previous studies of the yolk-sac of the 10-mm. pig embryo[7] and of the mongoose embryo[8] I have shown that the giant-cells of the yolk-sac arise from hemoblasts and may function as erythroblasts; that is, they may differentiate erythrocytes intracellularly. The present study shows that all of these cells involved in the hemoblast and giant-cell history contain metachromatic granules and that such cells may produce typical blood-platelets. Wright's work reveals identical conditions in the body blood-vessels of the guinea-pig embryo and in adult red bone-marrow. I can abundantly confirm Wright's conclu-

sion regarding these cells as progenitors of platelets in the case of the marrow of the femurs of the guinea-pig and the rabbit. The more complete evidence now permits the conclusion that the giant-cells are derived from hemoblasts, that they produce platelets wherever found, in yolk-sac, liver, and red marrow, and that they may at the same time function as multiple erythroblasts; that is, they are in fact hemogenic giant-cells in contrast with the osteoclastic giant-cells.

The foregoing leads to a closer analysis of the phenomenon of platelet formation by cells with metachromatic granules. The question resolves itself essentially into one regarding the significance of platelet formation by giant-cells ('megakaryocytes' and 'polykaryocytes'). An extensive microscopic study of the red bone-marrow of the femur of the frog—the details of which will be published elsewhere—reveals the following essential facts: All types of primitive lymphocyte derivatives (lymphocytes, thrombocytes, and granulocytes) may form pseudopods, which may constrict or segment to form platelet-like bodies. Thus the young hemoblasts and lymphocytes may produce hyaline bodies; the polymorphonucleated special (neutrophilic) granulocytes produce corpuscles with neutrophilic granules that is, elements suggestive of platelets; the thrombocytes, especially in the circulation, produce similar bodies; the basophilic granulocytes or mast-cells produce platelet-like bodies with coarse basophilic granules; the eosinophilic granulocytes produce platelet like bodies which may occasionally contain eosinophilic granules, but more generally lack granules hyaline bodies); and certain lymphocytes of the circulation which contain metachromatic granules may also produce by a similar method typical platelets.

The evidence from the study of the frog's marrow indicates that pseudopod formation and constriction is a common characteristic of lymphocytes and their leucocyte derivatives. Blood-platelets are accordingly a by-product of this phenomenon, and genetically belong in the same class with hyaline bodies and the cytoplasmic fragments of neutrophilic, basophilic, and eosinophilic granulocytes.

The suggestion presents itself that pseudopod segmentation and localized cytoplasmic fragmentation may to some extent be related to the nuclear amitosis also characteristic of these cells. It seems a reasonable assumption that the fundamental factors which cause a relative increase of nuclear substance by nuclear fission operate also to the same end by a decrease in the amount of cytoplasm by pseudopod constriction. The metabolic requirements as expressed in the nucleo-cytoplasmic relationship could conceivably be met either by increase of nuclear surface or by decrease of cytoplasmic volume, or still more effectively by a combination of both processes.

In the frog's marrow no naked nuclei seem to occur. In the circulation, however, naked nuclei of thrombocytes occur. The latter phenomenon alone seems to place these cells closer to the megakaryocytes of mammalian marrow than are the amphibian neutrophilic granulocytes. However, no strict homology obtains between the spindle cells or between the neutrophilic leucocytes and the megakaryocytes. In the frog's marrow occasional mononucleated giant-cells occur; they result from hypertrophy of certain primitive lymphocytes. These are the true homologues of the hemogenic giant-cells (mononucleated, polymorphonucleated, and polynucleated) of mammalian red marrow. These few giant-cells of the frog's marrow also contain metachromatic granules during later stages and may produce platelet-like bodies by pseudopod constriction.

As above described, platelets arise also by another method from the hemogenic giant-cells of mammalian red marrow, namely, by a process of fragmentation of larger areas of peripheral cytoplasm. This mode of origin was recognized also originally by Wright and subsequently by Downey. But neither seems to have grasped the full implication of the phenomenon. A similar double mode of origin of platelets is exemplified also in the case of the hemoblast and the giant-cells of the yolk-sac. In his report on the origin of platelets from megakaryocytes Wright paid special attention to the segmenting pseudopods; Downey, as judged by his illustrations, saw principally the other mode, namely, origin by cytoplasmic fragmentation. A

comparison of their illustrations (fig. 14, Wright;[11] figs. 15, Downey[4]) shows that the first mode is associated with healthy nuclear condition, the latter with a degenerating nucleus. The platelet progenitor of the yolk-sac of the 12-mm. pig embryo shows the same nuclear conditions associated with these two modes of platelet formation. It is obvious that either mode leads to practically the same morphologic result, namely, small globules of slightly basophilic cytoplasm containing metachromatic granules.

In the light of these observations, platelet formation is apparently simply a by-product of cytoplasmic fragmentation of certain cells with metachromatic granules. A careful study of the marrow of the femur of the rabbit and of the guinea-pig has convinced me of the accuracy of this interpretation in part as applied also to these marrows. The evidence, then, from a comparative study of the marrows of guinea-pig, rabbit, and frog, and of the yolk-sac of the pig embryo, consistently points to the same conclusion, namely, that a giant-cell is a hypertrophied hemoblast, that it produces platelets as a by-product of apparently normal pseudopod formation and constriction—a process perhaps related to metabolic conditions as expressed in the nucleo-cytoplasmic relationship—and of cytoplasmic fragmentation, and that under certain conditions in a multinucleated form the giant-cell may function as a multiple erythroblast.

That the multinucleated giant-cell arises from the polymorphonucleated megakaryocyte by a process of separation of the lobules of the 'basket' nucleus can be readily demonstrated in the marrow of the rabbit and of the guinea-pig. Moreover, in the red marrow of the guinea-pig the polynucleated types predominate, while in the marrow of the rabbit the polymorphonucleated are by far the most common forms of giant-cells.

It seems desirable, finally, to attempt to bring the foregoing morphologic data into relation with the mechanism of coagulation. According to Howell,[5] clotting of blood plasma and of lymph involves the coöperation of four elements: 1, fibrinogen and 2, antithrombin, both present in both lymph and plasma;

3, prothrombin, liberated by blood-platelets and by lymphocytes and 4, thromboplastin, elaborated by platelets, lymphocytes and tissue cells generally, and operating to neutralize antithrombin. Though the lymph of the thoracic duct lacks platelets (Howell;[5] Jordan[6]) it nevertheless clots like blood plasma under similarly favorable conditions, only somewhat more slowly. The blood of birds, reptiles, and amphibia likewise clots in the absence of circulatory platelets; in these forms occur additional blood-cells, the thrombocytes or spindle cells, which appear to be analogues of the mammalian platelet. Spindle cells and mammalian platelets contain apparently identical metachromatic granules. Lymphocytes likewise contain a certain amount of similar granules. The source of the prothrombin of lymph would seem to be restricted, at least largely, to the preponderant lymphocytes. In non-mammalian bloods, as for example that of frog, the prothrombin could apparently be liberated by the spindle cell or by the lymphocyte or by both. The evidence suggests that the specific source of the prothrombin is the metachromatic granule. The combined physiologic and morphologic data seem to indicate that the metachromatic granules of hemoblast, hemogenic giant-cell, lymphocyte, spindle cell, and free platelets, whether of giant-cell or spindle-cell origin, are functionally similar. This suggestion seems the more plausible in view of the fact that the lymphocyte, hemogenic giant-cell, and spindle cell are all direct and but relatively slightly differentiated derivatives of the hemoblast. The precise relationship of the cytoplasmic granules of the polymorphonucleated neutrophilic granulocytes of certain mammals and amphibia to the closely similar metachromatic ('azurophil') granules of the above-specified group of cells remains undetermined.

SUMMARY

1. In the blood spaces of the yolk-sac and of the liver of the 12-mm. pig embryo typical blood-platelets occur in large numbers. They are produced by the primitive lymphocytes or hemoblasts and their giant-cell derivatives, occasionally also by

endothelial cells in process of differentiation into hemoblasts and separation from the vessel wall. The mode of platelet formation is twofold: *a*, by segmentation of pseudopods, and *b*, by fragmentation of larger portions of cytoplasm. All of these cells contain a homogeneous, slightly basophilic substratum filled with fine spheroidal metachromatic granules. The smaller mononucleated cells correspond with those described by Wright as megakaryocyte 'forerunners' in the guinea-pig embryo and in the red bone-marrow of certain mammals. Cytoplasmic fragmentation (disintegration) is associated with abnormal (or senile) nuclear conditions and leads to naked nuclei. Pseudopod segmentation is apparently a common phenomenon of normal lymphocytes and their leucocyte derivatives, and may be a method of maintaining the nucleo-cytoplasmic relationship at an optimum, an end aided also by the nuclear amitosis characteristic of these cells.

2. The giant-cells are essentially hypertrophied hemoblasts and in the yolk-sac may function as multiple erythroblasts.

3. Blood-platelet formation appears to be a by-product both of the normal activity and of the disintegration of potentially erythrocytogenic giant-cells.

4. The true amphibian homologue of the mammalian 'megakaryocyte' is not the thrombocyte, but a mononucleated giant-cell derived by hypertrophy from a primitive lymphocyte or hemoblast. Spindle cells of ichthyopsid and sauropsid bloods, and platelets of mammalian bloods apparently have a similar function in relation to thrombus formation; therefore they may be considered analogous elements, but no strict homology obtains between them.

5. Blood-platelets occur in the pig embryo coincidentally with the appearance of primitive lymphocytes, or yolk-sac hemoblasts, with metachromatic granules.

6. The evidence suggests that the seat of the prothrombin is the metachromatic granule, whether of hemoblast, megakaryocyte, spindle cell, lymphocyte, or platelet origin.

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